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THE UNIVERSITY OF ALBERTA

ON SOME METABOLITES OF MEDICINAL PLANTS

BY

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A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH IN
PARTIAL FULFILMENT FOR THE DEGREE OF DOCTOR OF PHILOSOPHY
DEPARTMENT OF CHEMISTRY

EDMONTON, ALBERTA
(SPRING 1989)

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DATE Nov. 4/88

To Magodi

ABSTRACT

Chemical studies on four medicinally important plants, *Lycopodium obscurum* L., *Mentha arvensis* L., *Geranium viscosissimum* Fisch. and Meg., and *Eriogonum umbellatum* Torr. have been carried out.

The minor alkaloids of the basic extract of *L. obscurum* have been investigated. Twenty alkaloids have been isolated. These include the previously reported bases, lycopodine (**6a**), clavolonine (**6b**), flabelliformine (**6c**), dihydrolycopodine (**7a**), acetyldihydrolycopodine (**7b**), β-lofoline (**7c**), lycofoline (**8**), lycodine (**10**), flabellidine (**11**), and des-N-methyl-α-obscurine (**12c**), as well as an unidentified alkaloid **53**. The first part of this thesis describes the chemical and physical methods employed in the structural elucidation of nine new bases: des-N-methyl-β-obscurine (**12d**), obscurinine (**13**), iso-obscurinine (**14**), lobscurinol (**27**), epilobscurinol (**29**), acetyllobscurinol (**30**), hydroxypropyllycodine (**36**), lyconnotinol (**38**), and acrifolinol (**43**). The structural elucidation of an unidentified base designated L17 (**47**) previously isolated from *L. obscurum* is also discussed.

It is proposed that obscurinine (**13**) and iso-obscurinine (**14**) are artefacts derived by addition of ammonia to appropriate precursors. Evidence in support of this hypothesis is presented. Hydroxypropyllycodine (**36**) is the first encountered Lycopodium alkaloid with a nineteen carbon skeleton and is biogenetically interesting. A possible biogenesis of hydroxypropyllycodine is discussed.

In Canadian native medicine, *M. arvensis* is used to treat various illnesses including heart diseases and *G. viscosissimum* and *E. umbellatum* are used in the treatment of infectious diseases. The chemical investigation of these plants including biological screening studies of the latter two species are presented in the second part of this thesis.

ACKNOWLEDGEMENTS

The author is indebted to:

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Last, but not least, the close friends whose spiritual support made this work easier.

TABLE OF CONTENTS

Chapter Page

I. **Introduction** 1

II. ***Lycopodium obscurum*** 8

III. ***Mentha arvensis*** 98

IV. ***Geranium viscosissimum*** **and** ***Eriogonum umbellatum*** 113

V. **Experimental** 132

- A. General 132
- B. *Lycopodium obscurum* 133
- C. *Mentha arvensis* 164
- D. *Geranium viscosissimum* and *Eriogonum umbellatum* 174

VI. **Bibliography** 189

LIST OF TABLES

Table Page

1. Lycopodium alkaloids skeleton types. 2
2. GC / MS for authentic samples of Lycopodium alkaloids. 10
3. ^{1}H nmr spectra of obscurinine (13) and iso-obscurinine (14). 15
4. ^{13}C nmr spectra of obscurinine (13) and iso-obscurinine (14). 16
5. ^{1}H nmr decoupling experiments with obscurinine (13). 28
6. ^{1}H nmr nOe experiments with obscurinine (13). 29
7. ^{1}H nmr spectra of lobscurinol (27), acetyllobscurinol (30), and acetylepilobscurininol (31). 41
8. ^{13}C nmr spectra of lobscurinol (27), acetyllobscurinol (30), and lobscurinine (28). 42
9. ^{1}H nmr decoupling experiments with lobscurinol (27). 46
10. ^{1}H nmr nOe experiments with lobscurinol (27). 47
11. Comparison of some ^{1}H nmr spectral data of lobscurinol (27) and acetyllobscurinol (30) with that of lycoplegmarine (32) and acetyllycoplegmarine (33). 50
12. ^{1}H nmr spectra of lycodine (10), hydroxypropyllycodine (36), and diacetylhydroxypropyllycodine (37). 54
13. ^{13}C nmr spectra of lycodine (10), hydroxypropyllycodine (36), and diacetylhydroxypropyllycodine (37). 55
14. ^{13}C nmr spectra of des-N-methyl-β-obscurine (12d) and huperzine B (2). 65
15. ^{1}H nmr nOe experiments with monoacetyllyconnotinol (39). 74
16. ^{13}C nmr spectra of lyconnotine (41), lyconnotinol (38), monoacetyllyconnotinol (39), and diacetyllyconnotinol (42). 79

17. ^{1}H nmr nOe experiments with acrifolinol (**43**). 85

18. ^{13}C nmr spectra of acrifolinol (**43**) and lycofoline (**8**)..............................89

19. ^{1}H nmr decoupling experiments with O-acetylannofoline (**47**)...................92

20. ^{13}C nmr spectra of α-lofoline (**45**), dehydro-α-lofoline (**46**), O-acetylannofoline (**47**), and dihydro-O-acetylacrifoline (**48**). 97

21. ^{1}H nmr decoupling experiments with lactone (**54**)............................... 101

22. Comparison of methyl group signals in the ^{1}H nmr spectra of 3β-acetyloleanolic acid (**60**) and methyl 3β-acetylursolate (**64**) with literature data. 109

23. ^{1}H nmr spectra of methyl 3β-acetylursolate (**64**) and methyl 3β-acetylpomolate (**66**).. 112

24. Antibacterial screening of crude extracts of *G. viscosissimum* and *E. umbellatum* and pure metabolites **69** and **81** 115

25. ^{13}C nmr spectra of **81** and **83** 127

26. ^{1}H nmr spectra of **82** and **84**.. 129

27. Comparison of some ^{1}H nmr spectral data for **78** and **88**. 131

28. Preliminary separation of the crude base extract of *L. obscurum*. 140

29. The crude extracts of *G. viscosissimum* and *E. umbellatum* 176

LIST OF FIGURES

Figure Page

1. GC / MS for the sample of *L. obscurum* from eastern Canada. 11
2. GC / MS for the sample of *L. obscurum* from western Canada. 12
3. COSY 90 spectrum of obscurinine (13).. 20
4. A slice from the COSY 90 spectrum of obscurinine (13). 22
5. NOESY spectrum of obscurinine (13)... 23
6. Expansion of the NOESY spectrum of obscurinine (13)........................ 24
7. ^{1}H-^{13}C COSY spectrum of obscurinine (13).. 25
8. COSY 90 spectrum of iso-obscurinine (14). 33
9. COSY 90 spectrum of lobscurinol (27). 44
10. COSY 90 spectrum of hydroxypropyllycodine (36)............................ 57
11. COSY 90 spectrum of diacetylhydroxypropyllycodine (37).................. 60
12. Expansion of COSY 90 spectrum of diacetylhydroxypropyllycodine (37). . 61
13. COSY 90 spectrum of monoacetyllyconnotinol (39). 70
14. Slices from the COSY 90 spectrum of monoacetyllyconnotinol (39)......... 71
15. NOESY spectrum of monoacetyllyconnotinol (39). 73
16. ^{1}H-^{13}C COSY of monoacetyllyconnotinol (39).................................. 80

LIST OF SCHEMES

Scheme Page

1. ^{1}H-^{1}H chemical shift correlations obtained from the COSY 90 spectrum of obscrurinine (13) 19
2. Low molecular weight ions in the hreims of obscurinine ~~(13).~~ 27
3. ^{1}H-^{1}H chemical shift correlations obtained from the COSY 90 spectrum of iso-obscurinine (14) 32
4. A summary of the biogenesis of the flabellidane, phlegmarane, and cernuane Lycopodium alkaloid skeletons 35
5. A summary of the biogenesis of the lucidulane, lycopodane, fawcettimane, fawcettidane, and related Lycopodium alkaloid skeletons 36
6. ^{1}H-^{1}H chemical shift correlations obtained from the COSY 90 spectrum of lobscurinol (27) 43
7. Fragmentation in the hreims of lobscurinol (27) 48
8. ^{1}H-^{1}H chemical shift correlation obtained from the COSY 90 spectrum of hydroxypropyllycodine (36) 56
9. Fragmentation in the hreims of hydroxypropyllycodine (36) 58
10. A summary of the biogenesis of some Lycopodium alkaloids 62
11. ^{1}H-^{1}H chemical shift correlations obtained from the COSY 90 spectrum of monoacetyllyconnotinol (39) 69
12. Fragmentation in the hreims of monoacetyllyconnotinol (39) 77
13. A possible biogenesis of lyconnotinol (38) from lycofoline (8) or acrifolinol (43) 78
14. ^{1}H-^{1}H chemical shift correlations obtained from the COSY 90 spectrum of acrifolinol (43) 82
15. ^{1}H-^{1}H chemical shift correlations obtained from the COSY 90 spectrum

of lycofoline (**8**). 87

16. ^{1}H nmr coupling pattern of lactone **54** 100

17. Fragmentation in the hreims of Δ^{12} oleanene and ursene triterpene acids. ... 105

18. Fragmentation in the hreims of compound **76**. 122

19. Extraction of *L.obscurum* in a Soxhlet apparatus. 136

20. Re-extraction of the crude bases of *L. obscurum* with tartaric acid. 137

21. Extraction of *L. obscurum* with tartaric acid. 138

22. Isolation of compounds **7b**, **11**, **13**, **14**, **30**, and **36**. 141

23. Isolation of compounds **6b**, **7c**, **8**, **38**, and **43** 150

24. Isolation of compounds **7a**, **12d**, and **53** 158

25. Extraction of *M. arvensis* by percolation with benzene. 165

26. Extraction of *M. arvensis* in a Soxhlet apparatus. 166

27. Extraction of *G. viscosissimum* and *E. umbellatum* in a Soxhlet apparatus.. 175

28. Re-extraction of the crude bases of *E. umbellatum* 181

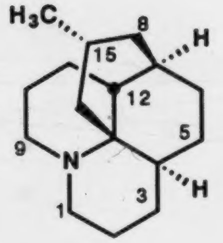
29. Isolation of compounds **74**, **81**, **83**, **85**, and **87** 184

I. Introduction.

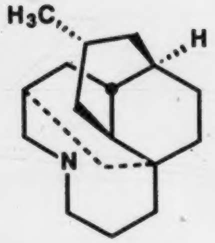
Lycopodium alkaloids have a long history dating back to 1881 when the first alkaloid, presumably lycopodine, was isolated from *Lycopodium complanatum*[1]. The presence of alkaloids in the order Lycopodiales is quite general. Alkaloids have been detected and isolated in all Lycopodium species studied to the present time. These alkaloids have been well reviewed[2,3]. The most recent review covers literature to the beginning of 1985[3c]. It has been noted that about 10% of the Lycopodium species have been studied and over 100 alkaloids distributed among approximately 22 skeletal types are known. Since 1985 a few new alkaloids have been added to the previous list. The Lycopodium alkaloid skeleton types may be categorized into four groups: the $C_{16}N$ group, the $C_{16}N_2$ group, the $C_{27}N_3$ group, and a group of alkaloids with other than 16 or 27 carbon atoms. These groups are shown in table 1. Early work in these laboratories contributed to the structure elucidation of many of the known alkaloids. Our renewed interest in the Lycopodium alkaloids was stimulated by a recent report on the use of Lycopodium alkaloids in treating Alzheimer's disease (AD).

Alzheimer's disease is a neurological disorder which affects cholinergic neurons[6,7]. The major symptoms of the disease are memory loss, disorientation, and deterioration of intellectual ability. The disease is common among the elderly, but the symptoms are not limited to the aged. There is no positive clinical test for Alzheimer's disease and, to date, diagnosis has relied upon direct comparison at autopsy of brain tissue from normal individuals with that of patients with Alzheimer's disease. It has been observed that the activities of two enzymes, cholineacetyltransferase (ChAT) and acetylcholinesterase (AChE) are greatly reduced in an Alzheimer's brain. ChAT catalyses the synthesis of acetylcholine and is found only within neurons which use acetylcholine

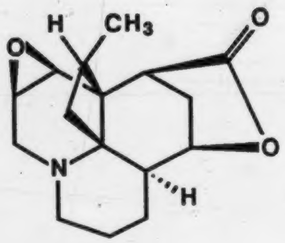
Table 1 $C_{16}N$ Skeletons :


H3C
8
H
15
12
5
9
N
3
H
1

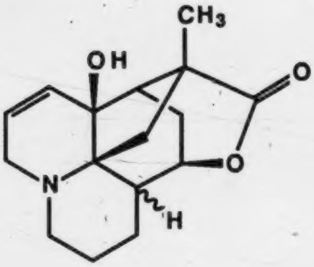
Lycopodane



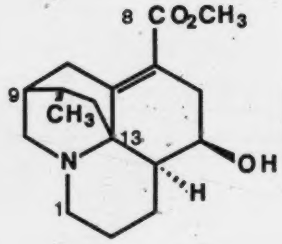
Alopecurane



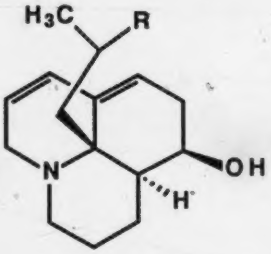
Annotinine



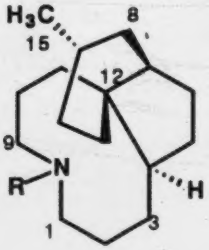
Annotine


8
CO2CH3
9
CH3
13
N
OH
H
1

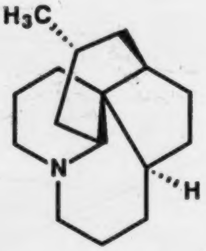
Annopodine



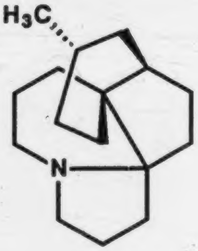
Lyconnotene


H3C
8
15
12
9
R
N
H
1
3

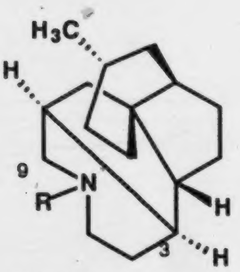
Fawcettimane



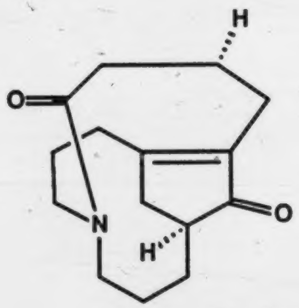
Fawcettidane



Serratinane

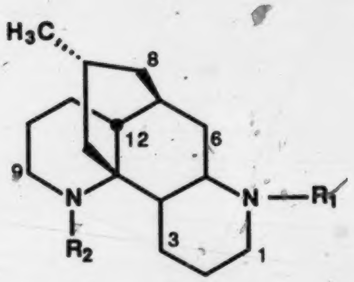

H3C
H
9
R
N
H
3
H

Magellanane

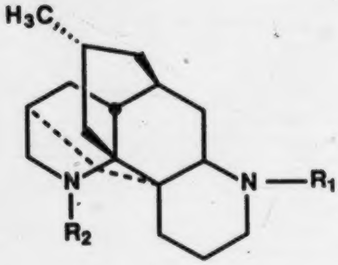


Phlegmariurine[4]

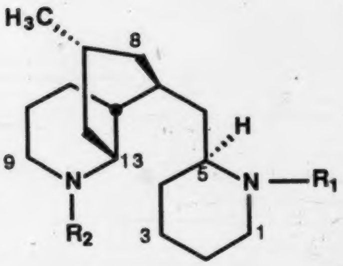
Table 1 cont. $C_{16}N_2$ Skeletons :


H3C
8
12
6
9
N
N
R1
R2
3
1

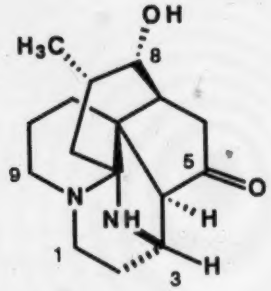
Flabellidane



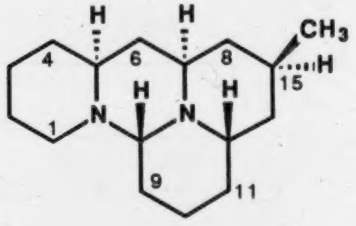
Fastigatane[5]


H3C
8
H
9
N
13
5
N
R1
R2
3
1

Phlegmarane


OH
H3C
8
9
5
N
O
NH
H
1
H
3

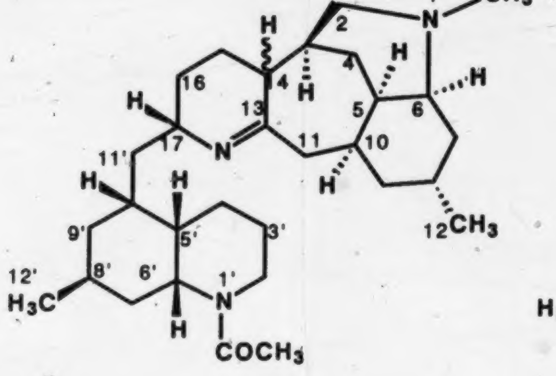
Base R


H
H
CH3
4
6
8
H
H
H
15
N
N
1
9
11

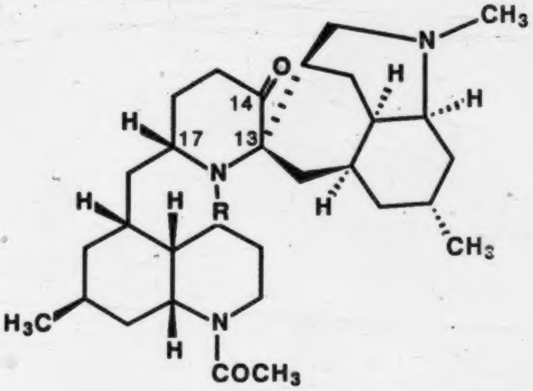
Cernuane

Table 1 cont.

$C_{27}N$ Skeletons :

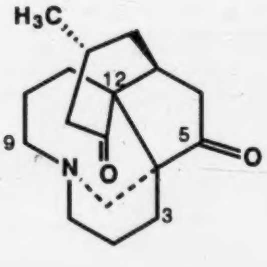

1
CH₃
N
H
2
4
H
16
14
H
H
H
13
5
6
H
17
N
11
10
11'
H
H
H
9'
5'
3'
12CH₃
12'
8'
6'
1'
H₃C
N
H
COCH₃

Lucidine

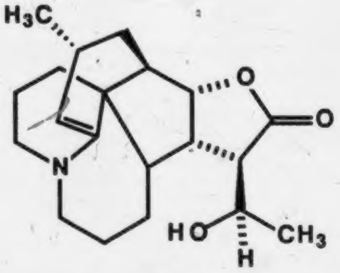

CH₃
N
O
H
14
H
H
17
13
N
R
H
H
H
CH₃
H₃C
N
H
COCH₃

Spirolucidine, R = H

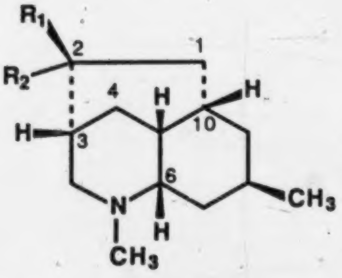
Skeletons other than C_{16} or C_{27}:


H₃C
12
9
5
O
N
O
3

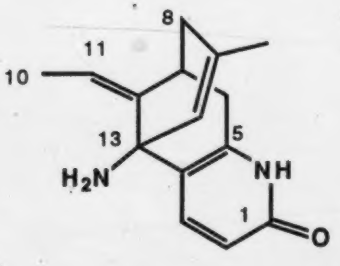
Lycoflexine


H₃C
O
O
N
HO
CH₃
H

Magastachine


R₁
2
1
R₂
4
H
H
H
3
10
6
CH₃
N
H
CH₃

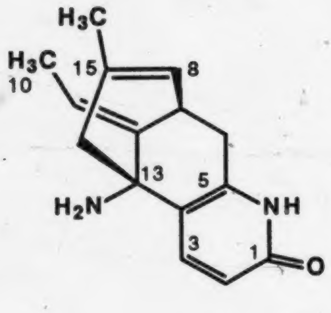
Lucidulane


8
11
10
13
5
H₂N
NH
1
O

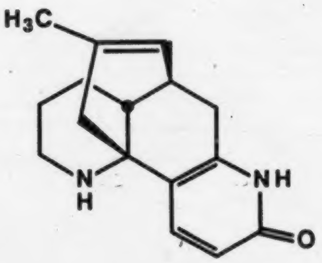
Selagine

as their neurotransmitter. AChE is heavily concentrated in neurons using acetylcholine but it is also found in some other cells. Although the pathogenesis of AD is not known, it is now well documented that acetylcholine is markedly deficient in brains of patients with AD.

To date, there is no cure for AD. A possible therapy is to restore acetylcholine levels. Acetylcholinesterase inhibitors such as physostigmane (eserine), tranquilizers, and vasodilators are used for symptomatic relief. On the other hand Chinese traditional medicine uses *Lycopodium serratum* Thunb to treat AD. Recently, a chemical investigation of *L. serratum* led to the isolation of the alkaloids huperzine A (1) and B (2)[8]. Huperzine A and B exhibit anticholinesterase activity in pharmacologic studies[9]. The use of huperzine A in the treatment of myasthenia gravis, Alzheimer's dementia and for the improvement of senile memory loss is currently under clinical investigation. Another report of the use of a Lycopodium plant in AD-related disorder dates back to the late 1940's, when the powdered plant of *L. selago* was used as a substitute for pilocarpine and eserine in glaucoma[10].

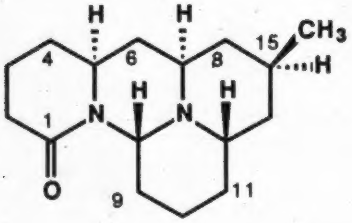


1

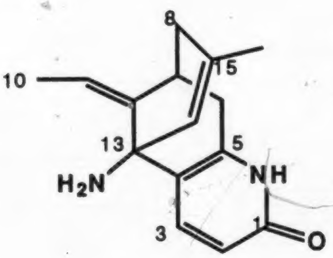


2

The activities of huperzine A and B in treating AD prompted us to conduct *in vitro* bioassays using samples from the large collection of Lycopodium alkaloids available to us. At the present time *in vitro* bioassays to correlate Lycopodium alkaloid skeletal type to cholinergic activity are in progress in our laboratories. Of the several alkaloids tested, cernuine (3) (*L. cernuum*) and selagine (4) (*L. selago*), present in the crude base extract of *L. selago*, represent two of the more active cholinesterase inhibitors *in vitro*. *In vivo* bioassays with these alkaloids are currently under investigation. These findings have stimulated our interest in the minor alkaloids of *L. obscurum*, since this plant contains alkaloids with a skeleton (flabellidane) similar to that of the huperzines. Early studies by Manske and Marion[11] and in these laboratories[12] had revealed *L. obscurum* to be a rich source of Lycopodium alkaloids of the flabellidane class. Previous studies on *L. obscurum* were not extensive. Only 8 alkaloids were reported. These include: lycopodine (**6a**), annotinine (**9**), lycodine (**10**), a molecular complex of α- and β-obscurine (**12a** and **12b**), and three alkaloids designated L13, L16, and L17. The first part of this thesis discusses results of the chemical investigation of the minor alkaloids of *L. obscurum*.



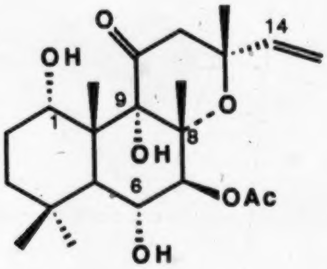
3



4

In the second part of this thesis, results of the chemical investigation of three plants, *Mentha arvensis var* (Labiatae), *Geranium viscosissimum*, and *Eriogonum umbellatum* (Polygonaceae) are discussed. These plants are indigenous to Canada and have been used in traditional medicine by Canadian native indians to treat various ailments. We were interested in studying the active principles of these plants.

In the first study, *M. arvensis*, a plant used to treat various ailments including heart diseases[13] was investigated. Our interest in this plant was stimulated by its use in treating heart diseases coupled with the observation that *Coleus forskohlii* Briq (Labiatae), which belongs to the same family as *M. arvensis*, is a rich source of diterpenes including forskolin (5)[14]. Forskolin is a novel iohotropic and blood pressure lowering agent[15]. Extracts of *M. arvensis* were initially screened for the presence of forskolin.



5

In another study several plants which are used by native indians to treat infected sores, swelling, coughs, colds, etc., were extracted and the extracts were tested for antibacterial properties. Extracts which showed significant activities were investigated chemically to identify the active principles. *Geranium viscosissimum*, used for treating colds and headaches[13a], and *Eriogonum umbellatum*, used for treating sores and inflammation[13a], are two such plants which showed significant activities against a variety of bacteria. In this thesis we report the isolation and identification of several metabolites of these plants including the biologically active components.

II. Lycopodium obscurum.

Recent indications that Lycopodium alkaloids may be useful in treating Alzheimer's disease(AD) have led us to reexamine the minor alkaloids of *L . obscurum* .

In 1944 Marion and Manske examined the basic extract of *L. obscurum* var *dedroideum* (Michx)[11] and reported the isolation of lycopodine (**6a**), a molecular complex of α- and β-obscurine (**12a** and **12b**), and three unidentified alkaloids designated L13, L16, and L17. The structures of α- and β-obscurine were determined during the 1960's in these laboratories[16], while L13 has been shown to be identical with lycopodine (**6a**)[17]. We have recently undertaken studies of L16 and L17 obtained from Marion and Manske's collection. Preliminary examination of alkaloid L16 revealed it to be a mixture of three alkaloids of molecular formulas* $C_{16}H_{25}N$ (231), $C_{16}H_{23}NO$ (245), and $C_{16}H_{25}NO$ (247). Due to the limited sample no attempt was made to isolate the component bases of L16. The structure of L17 is discussed later in this thesis. In 1960 the basic extract of *L. obscurum* L. was examined in these laboratories[12]. Lycopodine (**6a**), lycodine (**10**), annotinine (**9**), and several other carbonyl containing compounds were isolated and reported.

We began the reexamination of the alkaloids present in *L. obscurum* by comparing samples of *L. obscurum* from eastern (New Brunswick) and western (Alberta) Canada using gas-chromatography / mass-spectrometry (gc / ms) according to the method of MacLean[18]. This method provides a quick means of identifying alkaloids by molecular ion and fragmentation patterns, since much of the necessary information is available in the literature. Table 2 shows the gc / ms retention indeces, which are directly proportional to the retention times over a 20 minute period for a standard solution of by molecular ion and fragmentation patterns, since much of the necessary information is available in the literature. Table 2 shows the gc / ms retention indeces, which are directly proportional to the retention times over a 20 minute period for a standard solution of known Lycopodium alkaloids. The gc / ms traces for the crude alkaloid mixture from *L. obscurum* are shown in figures 1 (New Brunswick) and 2 (Alberta). The gc / ms traces show that the alkaloid content of *L. obscurum* from eastern and western Canada is very similar: lycopodine (**6a**) is the major alkaloid followed by clavolonine (**6b**) in each sample. There is a slight variation in composition and quantity of the minor alkaloids. Since the sample of crude bases for *L. obscurum* from eastern Canada was small, no attempt was made to isolate and identify the minor bases from this sample. The chemical investigation on the sample of *L. obscurum* from western Canada is described below.

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* Data from gc / ms.

Preliminary chromatography of the crude basic extract of *L. obscurum* from Alberta partitioned the bases into least polar (highest Rf), medium polar, and more polar fractions. Examination of these fractions led to the isolation of twenty bases. Ten of the bases isolated from the medium polar fractions are known compounds. These include: lycopodine (**6a**), clavolonine (**6b**), flabelliformine (**6c**), dihydrolycopodine (**7a**), acetyldihydrolycopodine (**7b**), β-lofoline (**7c**), lycofoline (**8**), lycodine (**10**), flabellidine (**11**), and des-N-methyl-α-obscurine (**12a**). The structures of these alkaloids were assigned on the basis of physical and spectral data, which was compared with literature data and data for authentic samples (see Experimental for the isolation procedure and spectral data).

Chromatographic separation of the least polar fractions afforded four new alkaloids (**13**, **14**, **30**, and **36**), while the more polar fractions afforded six new bases (**12d**, **27**, **29**, **38**, **43**, and **53**). The structure of each new compound has been determined except for base **53**, which is currently under investigation in this laboratory.

Table 2 GC / MS for authentic samples of Lycopodium alkaloids.

	Relative retention index	M+	Alkaloid identity
1	68	242	lycodine
2	124	249	dihydrolycopodine
3	134	247	lycopodine
4	160	261	acrifoline
5	178	263	flabelliformine
6	186	261	serratidine
7	276	303	O-acetylacrifoline
8	313	260	des-N-methyl-α-obscurine
9	330	307	α-lofoline
10	354	274	α-obscurine

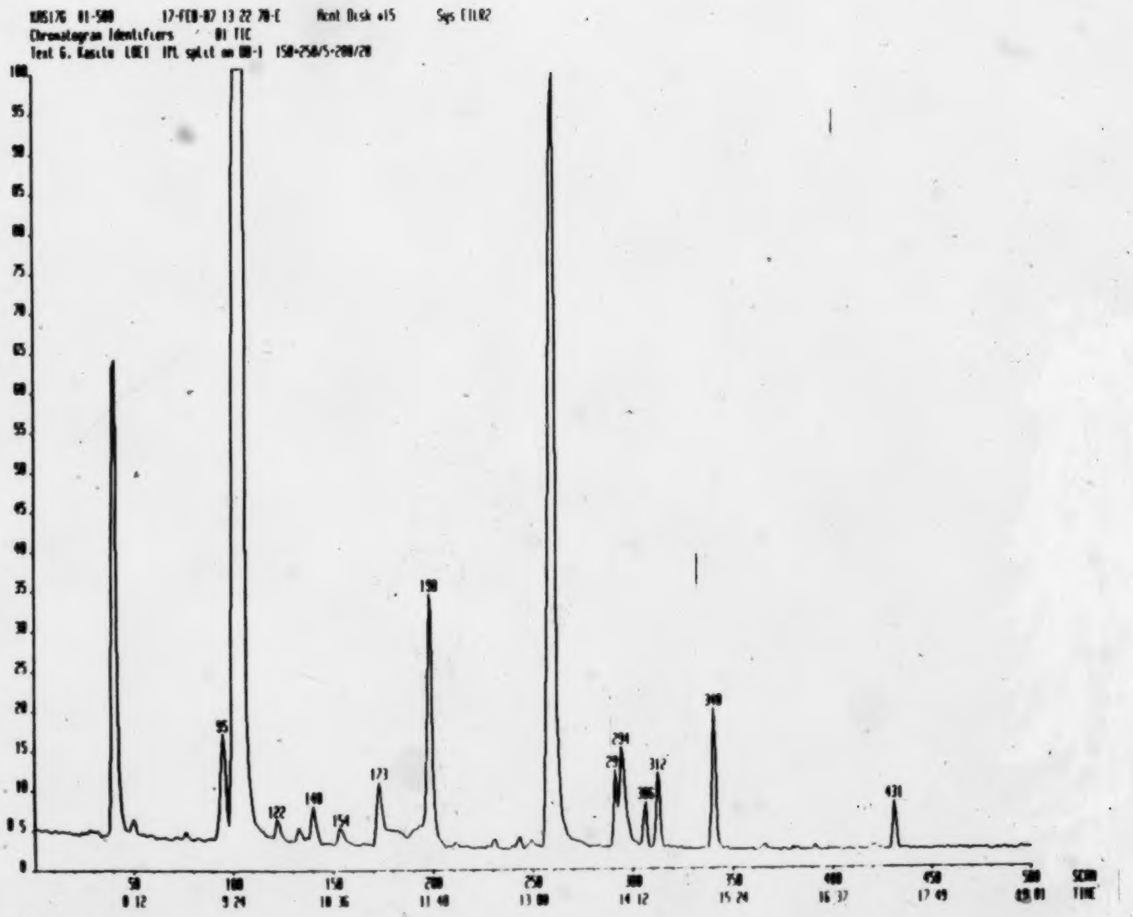


Figure 1. GC / MS for the sample of *L. obscurum* from eastern Canada.

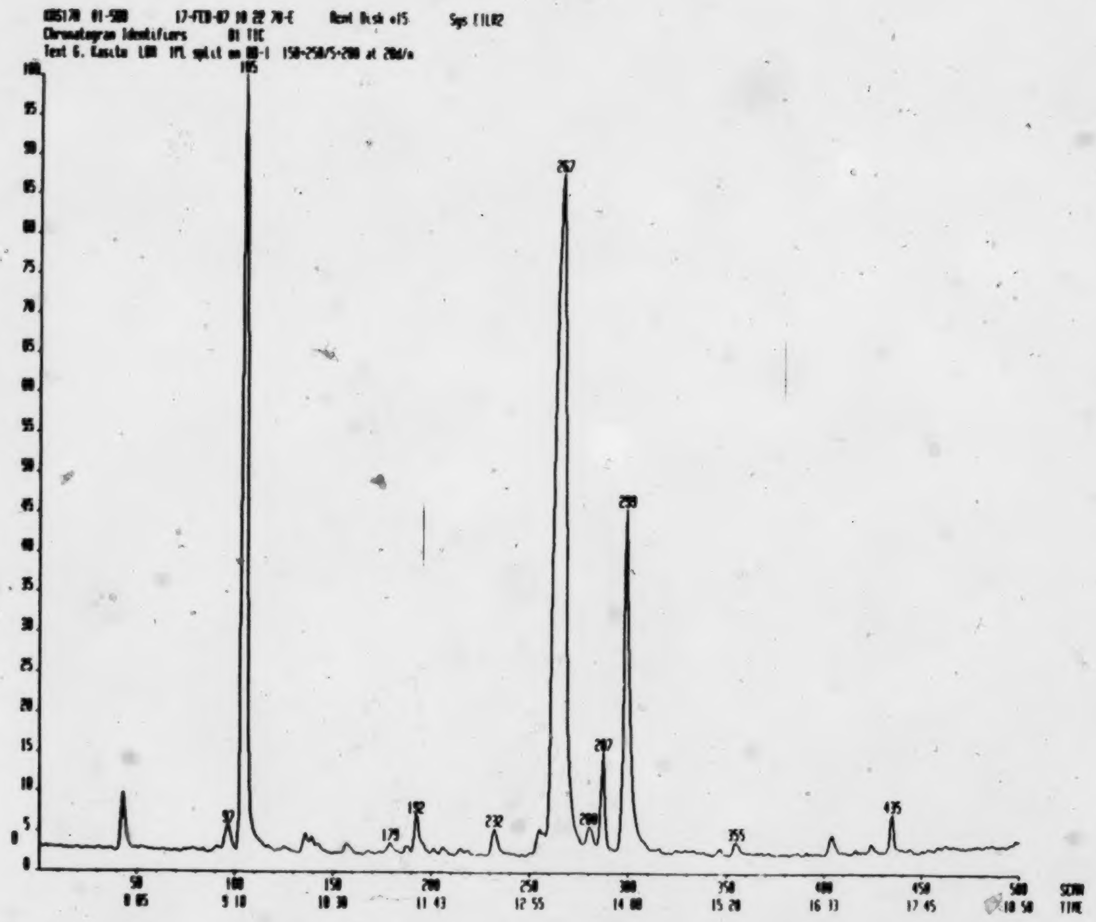
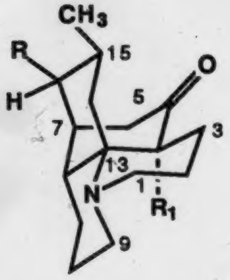
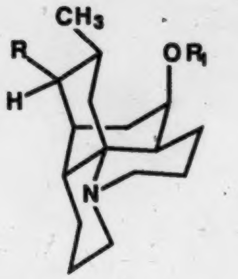


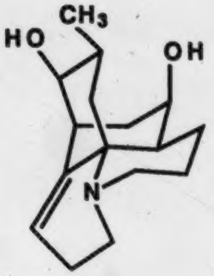
Figure 2. GC / MS for the sample of *L. obscurum* from western Canada.


CH3
R
15
O
H
5
7
3
13
N
1
R1
9

6a $R = R_1 = H$
6b $R = OH, R_1 = H$
6c $R = H, R_1 = OH$



7a $R = R_1 = H$
7b $R = H, R_1 = Ac$
7c $R = OH, R_1 = Ac$



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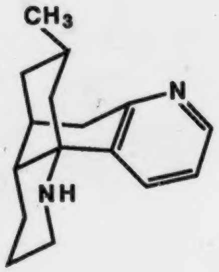
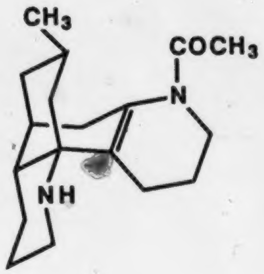
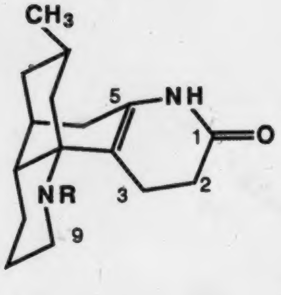


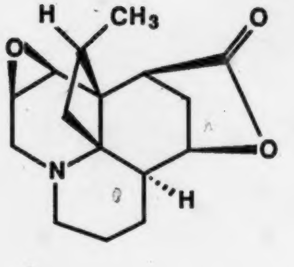
10


CH3
COCH3
N
NH

11


CH3
5
NH
1
O
NR
3
2
9

12a $R = CH_3$
12b $R = CH_3, \Delta^{2,3}$
12c $R = H$
12d $R = H, \Delta^{2,3}$



9

Obscurinine (13) and iso-obscurinine (14)

Bases 13 and 14, identified as obscurinine* and iso-obscurinine, respectively are isomeric. The molecular formula for each base ($C_{17}H_{24}N_2$, 272) was determined by high resolution electron impact mass spectrometry (hreims). Chemical ionization mass spectrometry (cims) confirmed the molecular weight.

The major isomer, obscurinine (**13**), on tlc has a higher Rf than lycopodine (**6a**). Base **13** crystallizes by evaporation from acetone / Skellysolve B as off white plates, mp 128-130°C, which are optically active. The Fourier transform infrared (ftir)[20] and ^{13}C nuclear magnetic resonance (nmr)[21] indicate a cyclopentanone (ftir: 1731 cm^{-1}; nmr: δ 220.3 (s)). In addition to the ketone group (δ 220.3), the ^{13}C nmr spectrum shows three other sp^2 hybridised carbons (δ 175.6 (s), 146.7 (s), and 119.1 (d)). Since there is only one oxygen in the molecular formula, the carbon-13 signal (δ 175.6) is assigned to an imine group. The ultraviolet (uv) absorption spectrum of base **13**, λ_{max} nm (logε) : 233 (4.04) suggests the presence of an α, β-unsaturated imine[22]. Its ftir (1645 and 1601 cm^{-1}) supports the presence of this chromophore. Since the molecular formula shows seven sites of unsaturation, this indicates that obscurinine possesses a tetracyclic skeleton.

Data obtained from one dimensional (1D) and two dimensional (2D) ^1H and ^{13}C nmr experiments with **13** was sufficient to complete the structural assignment for obscurinine.

In the 1D ^1H nmr spectrum (table 3), the signal at δ6.26 is assigned to an olefinic hydrogen. There are two methyl groups in the molecule as evidenced by absorption at δ2.29 (3H, s) and 1.85 (3H, s) consistent with a methyl group attached to a nitrogen

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* While our studies were underway Chandler *et al*[19] published the isolation of base 13, which they named obscurinine. We retain the name obscurinine.

Table 3 ^{1}H nmr spectra of obscurinine (13) and iso-obscurinine (14) ($CDCl_3$, 400 MHz).

	Chemical shift in δ, mult., J in Hz.		
H	**13**	**H**	**14**
14	6.26,brs,$W_{1/2}$=5	8	5.54,brs,$W_{1/2}$=10
3β	4.19,brs,$W_{1/2}$=8	3β	4.21,brs,$W_{1/2}$=7
4α	3.32,brs, $W_{1/2}$=3	4α	3.81,brt,3
8α	2.66,brddq,20,7.5,1	14a	3.00,brd,19
1a	2.54,ddd,13.5,13.5,3	14b	2.92,brd,19
7α	2.43,ddd,13.5,13.5,3	7α	2.67,brsext,6
9a	2.29,ddd,12,4,2	1a	2.59,brddd,14,14,3.5
6α	2.25,ddd,16.5,7.5,2	6α	2.44,ddd,18,8,2
17(3H)	2.20,s	9a	2.31,brddd,12,3,3
11a	2.18,m	9b & 11a	2.27-2.11,m
9b	2.13,m	17(3H)	2.21,s
8β	2.12,brd,20	1b & 2a/b	2.04-1.82,m
2a	1.99,dddd,13.5,13.5, 3, 3		
6β	1.97,dd,16.5,13.5	6β	1.87,dd,18,12
2b	1.96,m		
1b	1.90,ddd,13.5,3, 3		
16(3H)	1.85,brs,	16(3H)	1.78,brs,
10a/b &11b	1.55-1.40,m	10a/b &11	1.66-1.49

Table 4 ^{13}C nmr spectra of obscurinine (13) and iso-obscurinine (14) ($CDCl_3$,100.6 MHz).

	Chemical shift in δ.	
C	13	14
5	220.3 (s)	220.6 (s)
13	175.6 (s)	178.5 (s)
15	146.7 (s)	131.9 (s)
14	119.1 (d)	36.1 (t)
3	76.8 (d)	75.9 (d)
4	61.1 (d)	61.2 (d)
12	60.9 (s)	61.8 (s)
9	58.8 (t)	58.4 (t)
1	48.2 (t)	47.0 (t)
17	44.3 (q)	44.2 (q)
6	43.4 (t)	44.9 (t)
7	39.8 (d)	46.3 (d)
11	34.5 (t)	34.7 (t)
8	31.1 (t)	122.8 (d)
2	30.3 (t)	30.0 (t)
10	24.0 (t)	24.2 (t)
16	23.8 (q)	23.2 (q)

atom and an alkenic methyl group, respectively. There are no D_2O exchangeable hydrogens, thus the signal at δ4.19 must be due to a hydrogen *geminal* to a nitrogen atom. The remainder of the hydrogen signals (table 3) account for all the remaining hydrogens in the molecular formula.

The 1D ^{13}C attached proton test (APT) experiment (table 4) shows all the seventeen carbons and their multiplicities (11 quaternary / CH_2 signals and 6 inverted CH_3/ CH signals). A modified* ^{13}C APT experiment, in which only quaternary carbons absorb, indicates that only four of the eleven quaternary / CH_2 signals are quaternary (δ220.3, 175.6, 146.7, and 60.9). An unambiguous assignment was thus made for the signals at δ60.9 (quaternary) and δ58.8 (methylene). Since two methyl groups are observed in the ^1H nmr spectrum of obscurinine, four of the six CH_3 / CH signals must be due to methines (δ119.1, 76.8, 61.1, and 39.8).

The 2D hydrogen homonuclear correlation experiment is very informative. Two isolated spin systems I and II (scheme 1) were discerned from analysis of the COSY 90 spectrum (figure 3). Scalar couplings between hydrogens is indicated by double headed arrows (broken arrows indicate small couplings not readily observed at the instrumental conditions). The olefinic hydrogen (δ6.26) is coupled to the alkenic methyl (δ1.85) and an allylic methylene (δ2.66 and 2.12) groups. The methylene hydrogens are also coupled to the methyl group (δ1.85) and to a methine hydrogen (δ2.43), which is in turn coupled to a second methylene group (δ2.25 and 1.97). The large magnitude of the *geminal* couplings (J = 20 and 16.5 Hz) observed for the former (δ2.66 and 2.12) and latter (2.25 and 1.97) methylene group reflects their proximity to an sp^2 hybridised center[23]: α to the carbon-carbon double bond and α to the carbonyl group, respectively. The methylene hydrogens (δ2.25 and 1.97) are further coupled to a methine hydrogen

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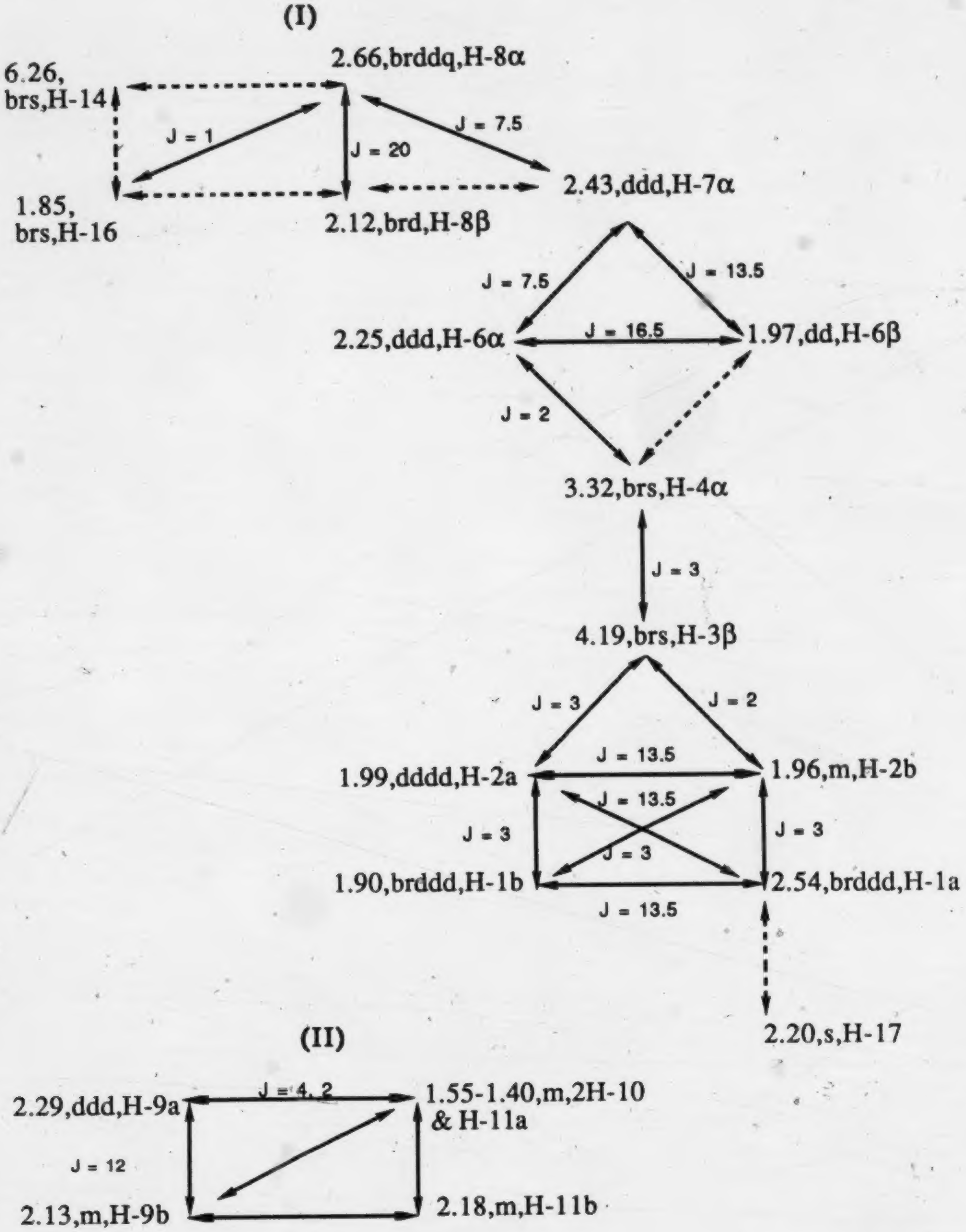
* The APT experiment was performed with variable delay of 0.007, half of the normal value.

(δ3.32) by a W pathway through the ketone group. This is consistent with the small magnitude of couplings observed (J = 2 Hz for the hydrogen resonating at δ2.25 and a smaller coupling, unobserved at the instrumental conditions, for its *geminal* partner (δ1.97)). The methine hydrogen (δ3.32) is also coupled to the methine hydrogen (δ4.19), which is in turn coupled to a methylene group (δ1.99 and 1.96), and so forth as summarized in spin system I. Partial structure A is consistent with this analysis.

The correlations outlined in spin system II are complicated somewhat by second order couplings. The hydrogen (δ2.29, ddd, J = 12, 4, 2 Hz) is coupled to two hydrogens resonating in a multiplet (δ1.55-1.40, 3H) and to a hydrogen whose signal is centered at *ca* δ2.13 (see slice in the COSY 90 spectrum centered at δ2.29, figure 4). Spin decoupling experiments in the ^{1}H nmr spectrum of base **13** (table 4) indicate that the *geminal* partner to the hydrogen (δ2.29) is the hydrogen which resonates at δ2.13. Upon irradiation of the three hydrogen multiplet (δ1.55-1.40), the hydrogen (δ2.29, ddd, J = 12, 4, 2 Hz) collapses to a doublet (J = 12 Hz). Thus the three hydrogen multiplet is assigned to a *vicinal* methylene group and to a methylene hydrogen β to the methylene group (δ2.29 and 2.13). The *geminal* partner to the β hydrogen resonates at *ca* δ2.18. The analysis of spin system II suggests the presence of partial structure B. This partial structure is verified by a nuclear Overhauser exchange spectroscopy (NOESY) experiment (figure 5). The solid lines show through space dipolar couplings between the hydrogens. Couplings are observed between all the *geminal* pairs as shown in the expanded NOESY contour plot, figure 6.

The ^{1}H-^{13}C heteronuclear correlation experiment (figure 7) reveals the carbon-hydrogen connectivities and confirms the ^{13}C APT experiment (table 4). Structure **13** was thus formulated for obscurinine.


(I)
6.26, brs,H-14
2.66,brddq,H-8α
J = 1
J = 20
J = 7.5
1.85, brs,H-16
2.12,brd,H-8β
2.43,ddd,H-7α
J = 7.5
J = 13.5
2.25,ddd,H-6α
J = 16.5
1.97,dd,H-6β
J = 2
3.32,brs,H-4α
J = 3
4.19,brs,H-3β
J = 3
J = 2
J = 13.5
1.99,dddd,H-2a
1.96,m,H-2b
J = 13.5
J = 3
J = 3
J = 3
1.90,brddd,H-1b
2.54,brddd,H-1a
J = 13.5
2.20,s,H-17
(II)
2.29,ddd,H-9a
J = 4. 2
1.55-1.40,m,2H-10 & H-11a
J = 12
2.13,m,H-9b
2.18,m,H-11b

Scheme 1. ^{1}H-^{1}H chemical shift correlations obtained from the COSY 90 spectrum of obscurinine (**13**).

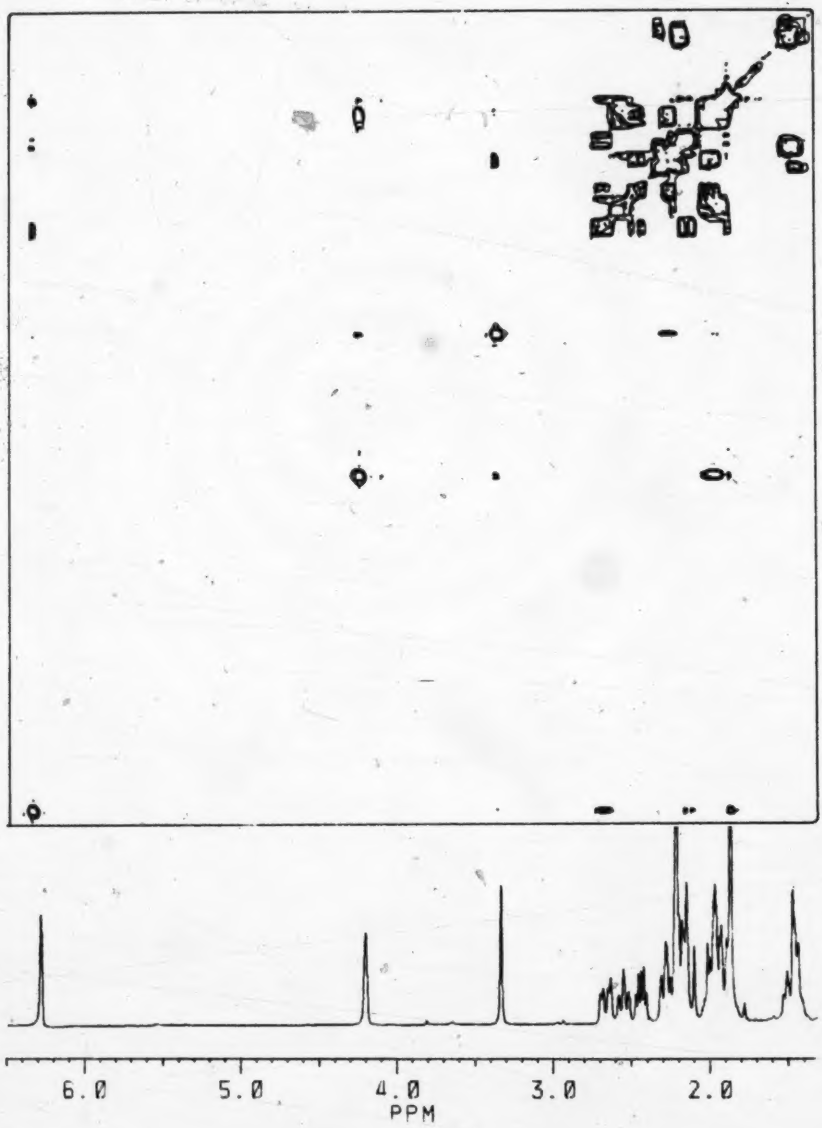
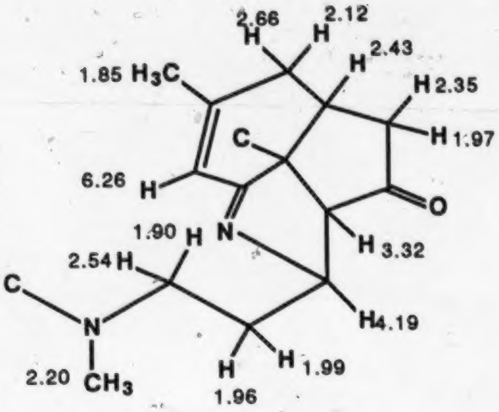
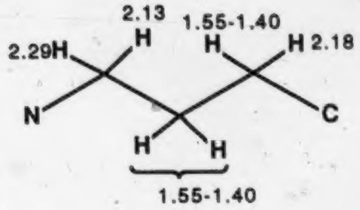


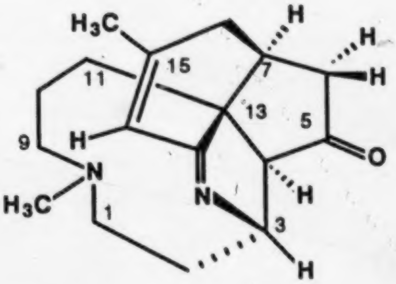
Figure 3. COSY 90 spectrum of obscurinine (13) ($CDCl_3$, 360 MHz).


2.66
2.12
2.43
1.85 H3C
H 2.35
H 1.97
C
6.26 H
N
O
1.90 H
2.54 H
H 3.32
C
N
H4.19
2.20 CH3
H 1.99
H
1.96

(A)


2.13
H
1.55-1.40
H
H 2.18
2.29H
N
C
H
H
1.55-1.40

(B)


H3C
H
H
H
11
15
7
13
5
9
H
N
O
N
H
H3C
1
3
H

13

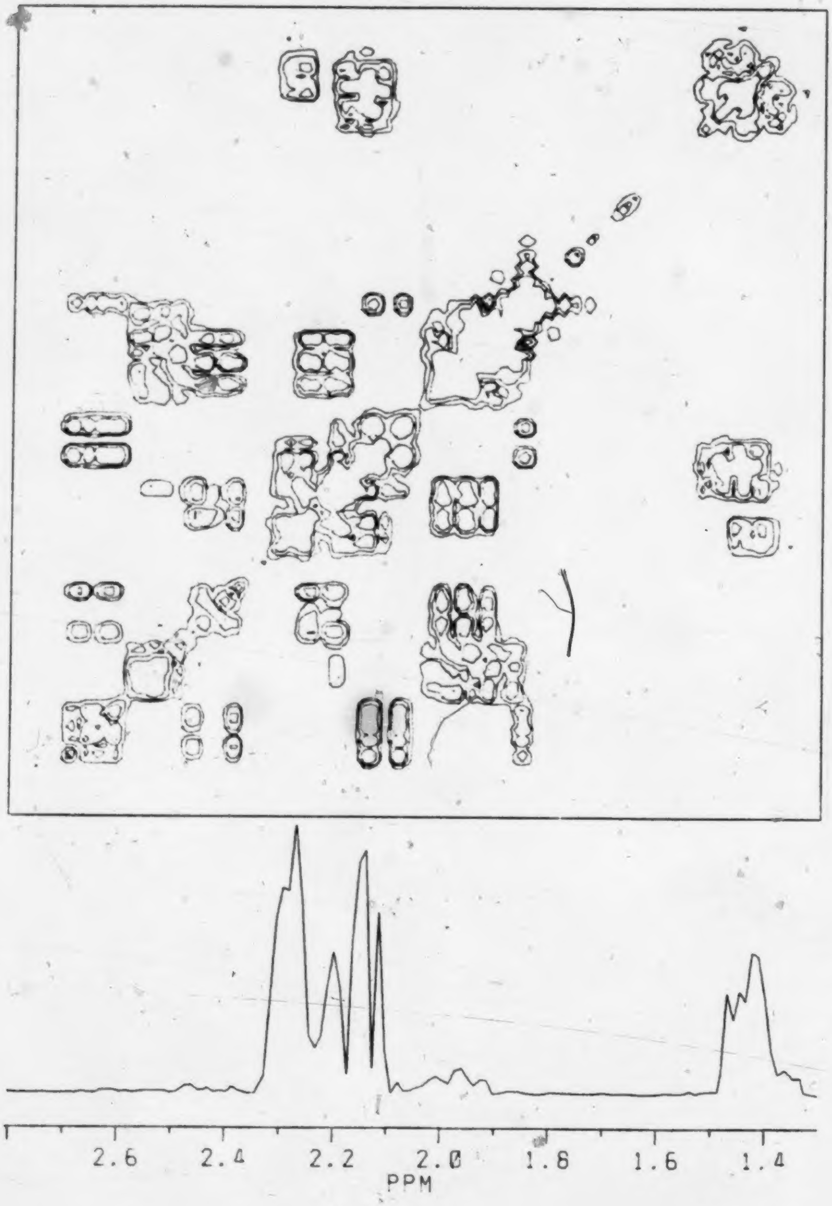


Figure 4. A slice centered at δ 2.29 from the COSY 90 spectrum of obscurinine (13) ($CDCl_3$, 360 MHz).

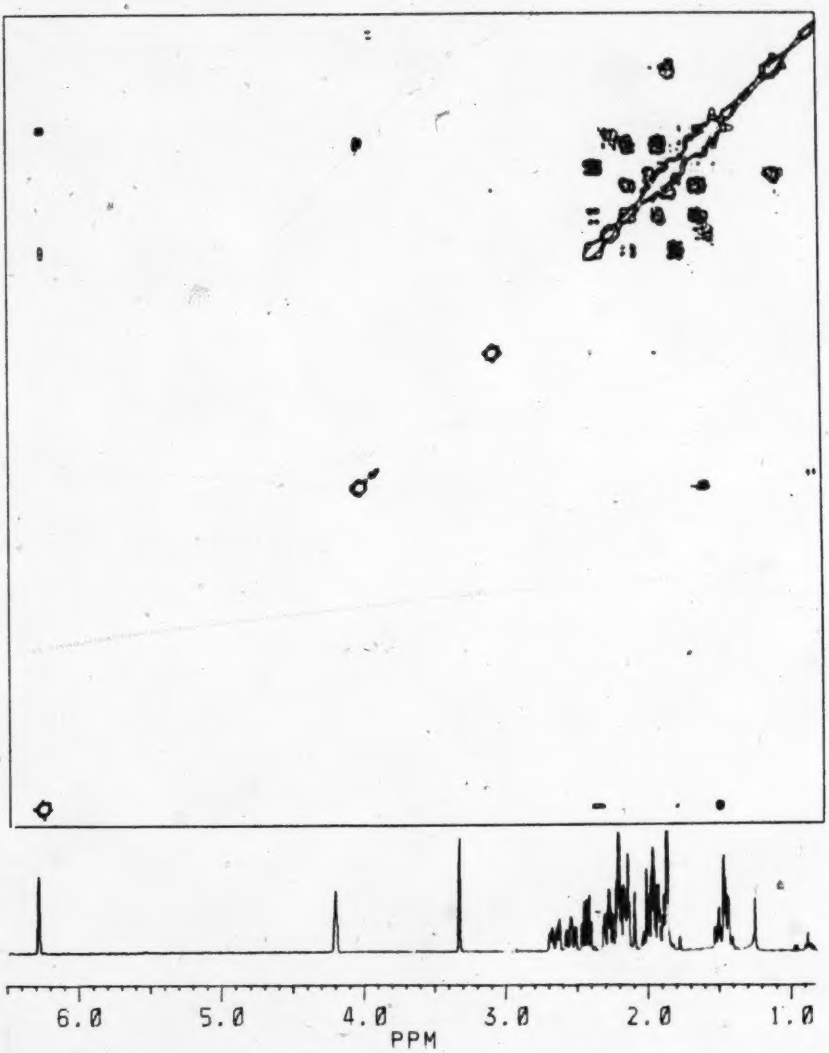


Figure 5. NOESY 45 spectrum of obscurinine (13) ($CDCl_3$, 400 MHz).

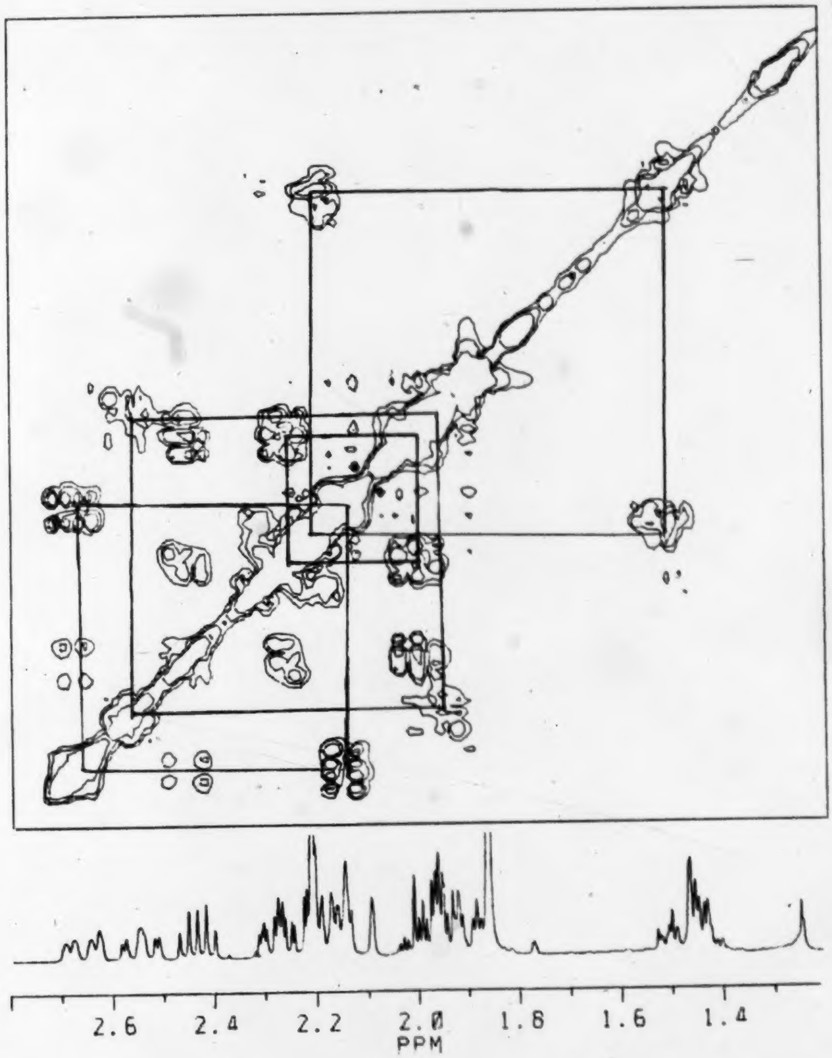


Figure 6. NOESY 45 expansion spectrum of obscurinine (13).

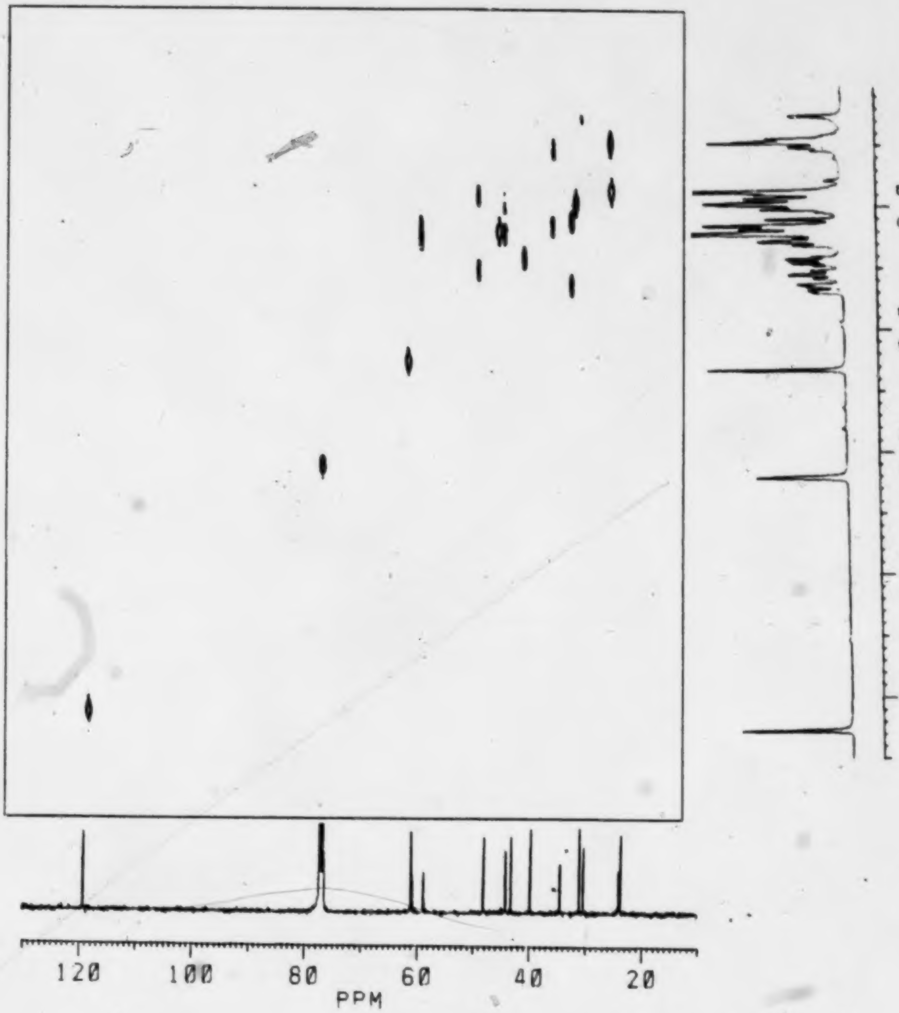


Figure 7. ^{1}H-^{13}C COSY spectrum of obscurinine (13) ($CDCl_3$, 400 MHz).

The fragmentation pattern in the hreims of base 13 is consistent with the structure assigned to obscurinine. The parent ion (m/z 272) is the base peak. Most of the fragment ions contain one or both nitrogen atoms in the molecular formula. The high molecular weight ions in the hreims of base 13 arise from loss of: a methyl, a carbonyl, ketene, ring A nitrogen containing ions, as well as ions from a combination of the above fragments (see Experimental). The low molecular weight ions are consistent with the fragmentation pattern as shown in scheme 2.

The relative stereostructure for obscurinine was proposed on the basis of spin decoupling experiments in the ^{1}H nmr (table 5) and in part by nuclear Overhauser (nOe) (table 6). The hydrogen at C-7 is α, *periplanar* to H-8α, and *antiperiplanar* to H-6β. Upon irradiation of H-7α (δ2.43, ddd, J = 13.5,7.5,7.5 Hz), H-8α (δ2.66, brddq, J = 20, 7.5,1 Hz) collapses to a broad doublet (J = 20 Hz), H-6α (δ2.25, ddd, J = 16.5,7.5,2 Hz) is perturbed*, while H-6β (δ1.97, dd, J = 16.5,13.5 Hz) collapses to a doublet (J = 16.5 Hz). The magnitude of the coupling constant between H-7α and H-6β, $J_{7\alpha,6\beta}$ = 13.5 Hz, is consistent with a *diaxial* coupling[24]. The hydrogen at C-4 is α. Upon irradiation of H-4α (δ3.32, brs, $W_{1/2}$ = 3 Hz), H-3 (δ4.19, brs, $W_{1/2}$ = 8 Hz) collapses to a broad double doublet (J = 3,3 Hz), while H-6α (δ2.25, ddd, J = 16.5,7.5,2 Hz) collapses to double doublet (J = 16.5,7.5 Hz) indicating that $J_{4\alpha,6\alpha}$ through the C-5 carbonyl group = 2 Hz. Such coupling would not be observed if H-4 was β-oriented. Steric considerations dictate that H-3 be α-oriented. The relative stereochemistry of obscurinine (13) was verified by an X-ray crystal structure obtained by Chandler and coworkers[19].

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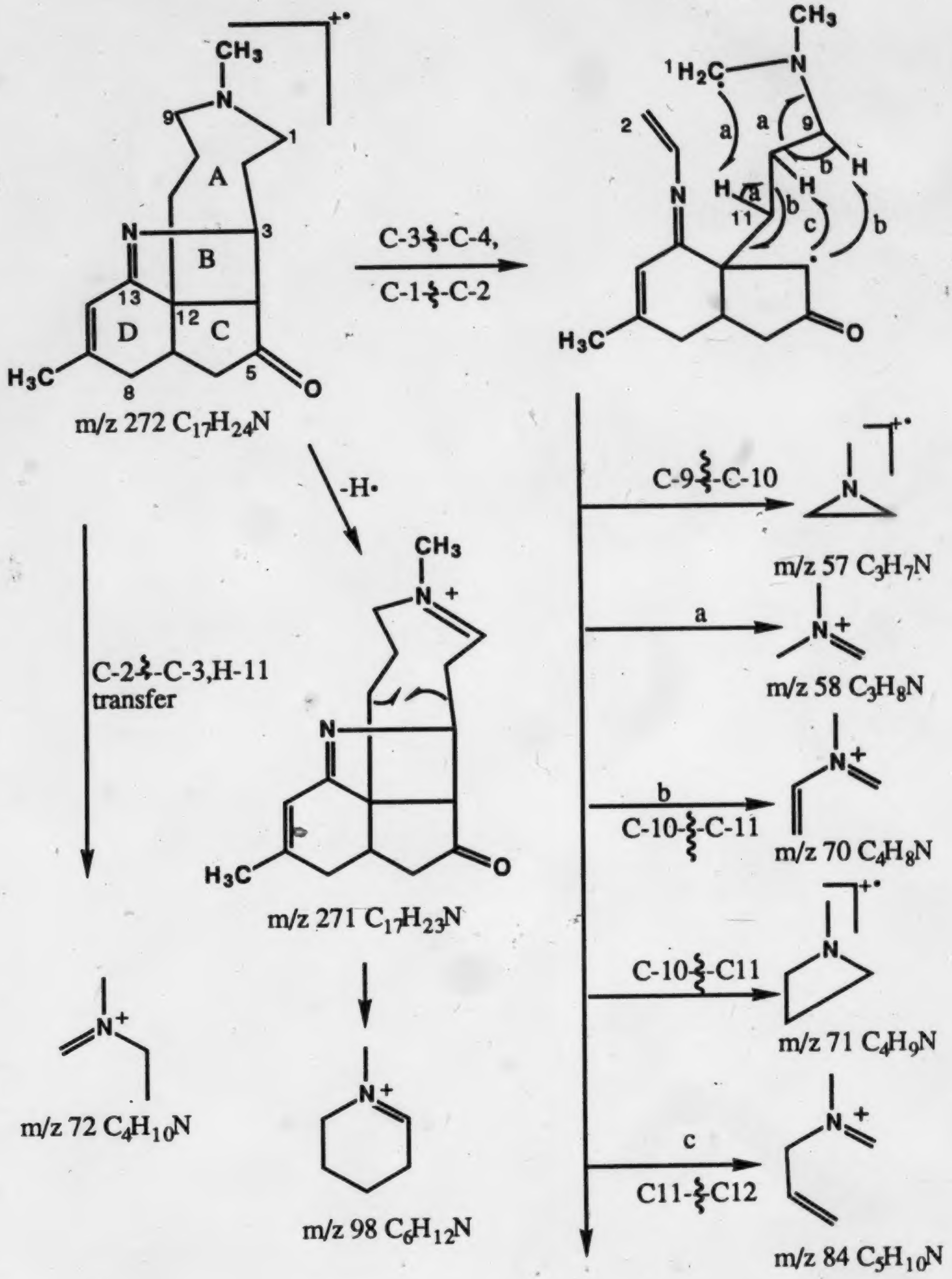
* Signal partially obscured by overlapping signals.


CH3
N
9
1
A
N
3
B
13
12
D
C
H3C
8
5
O
m/z 272 C17H24N
C-3-C-4,
C-1-C-2
CH3
1H2C
N
2
a
a
9
b
H
N
H
a
b
H
11
c
b
H3C
O
-H•
C-2-C-3,H-11
transfer
CH3
N+
N
H3C
O
m/z 271 C17H23N
m/z 72 C4H10N
N+
m/z 98 C6H12N
C-9-C-10
N
m/z 57 C3H7N
a
N+
m/z 58 C3H8N
b
C-10-C-11
N+
m/z 70 C4H8N
C-10-C11
N
m/z 71 C4H9N
c
C11-C12
N+
m/z 84 C5H10N

Scheme 2. Low molecular weight ions in the hreims of obscurinine (13).

Table 5 Decoupling experiments in the ^{1}H nmr spectrum of obscurinine (13) ($CDCl_3$, 400 MHz).

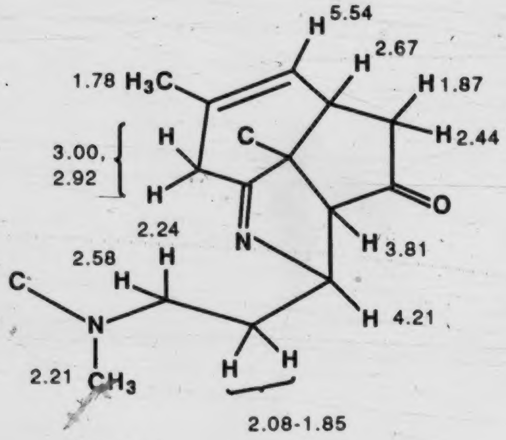
Chemical shift in δ, mult., J in Hz.		
Signal irradiated.	**Observed change.**	
6.25,brs,$W_{1/2}$=5(H-14)	2.66,brddd,20,7.5,1(H-8α)	dd,20,7.5
4.19,brs,$W_{1/2}$=8(H-3)	3.32,brs,$W_{1/2}$=3(H-4)	brd,2
	1.99,dddd,13.5,13.5,3,3(H-2a)	ddd, 13.5,13.5,3
	1.96,m(H-2b)	pert.
3.32,brs,$W_{1/2}$=3(H-4)	4.19,brs,$W_{1/2}$=8(H-3)	brdd,3,3
	2.25,ddd,16.5,7.5,2(H-6α)	dd,16.5,7.5
2.66,brddq,20,7.5,1(H-8α)	6.26,brs,$W_{1/2}$=5(H-3)	sharpened
	2.43,ddd,13.5,13.5,7.5(H-7)	dd,13.5,7.5
	2.12,brd,20(H-8β)	brs
2.54,brddd,13.5,13.5,3(H-1a)	1.99,dddd,13.5,13.5, 3,3(H-2a)	pert.
	1.96,m(H-2b)	"
	1.90,ddd,13.5,3,3(H-1b)	"
2.43,ddd,13.5,7.5,7.5(H-7)	2.66,brddq,20,7.5,1(H-8α)	brd,20
	2.25,ddd,16.5,7.5,2(H-6α)	pert.
	1.97,dd,16.5,13.5(H-6β)	d,16.5
1.55-1.40,m(H-11b &H-10a /b)	2.29,ddd,12,4,2(H-9a)	d,12
	2.18-2.13,m(H-9b &H-11b)	pert.

Table 6 ^{1}H nmr nOe experiments with obscurinine (13) ($CDCl_3$, 400 MHz).

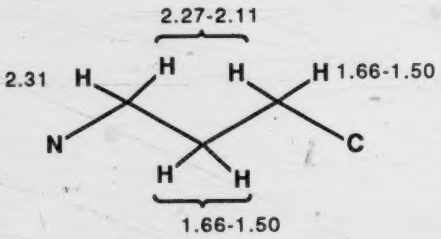
Chemical shift in δ.	
Signal saturated	**Signal enhanced(%nOe)**
6.26(H-14)	1.85(H-16)(1.2)
4.19(H-3)	3.32(H-4)(4.3)
3.32(H-4)	4.19(H-3)(4.3)
1.85(H16)	6.26(H-14)(9.5)

Iso-obscurinine (14) has a higher Rf (Rf 0.49, Skellysolve B:ethyl acetate; 4:1) than obscurinine (13). Iso-obscurinine crystallizes as colourless plates by evaporation from acetone / Skellysolve B, mp 106-107°C, and is optically active. Spectral analysis on iso-obscurinine revealed that the skeleton of obscurinine (13) is retained in iso-obscurinine (14) : the ^{13}C nmr spectrum indicates a cyclopentanone ring (δ 220.6), a C=N (δ 178.5), and a C=C (δ 131.9, s and 122.8, d) group and the ftir spectrum supports the presence of these groups (1734, 1649, and 1605 cm^{-1}, respectively). The seven sites of unsaturation indicated by the molecular formula (a carbonyl, an imine, and a C=C double bond) suggest that iso-obscurinine is tetracyclic. However, the imine and C=C functionalities in iso-obscurinine are isolated in accord with its inability to absorb uv light at 254 nm.

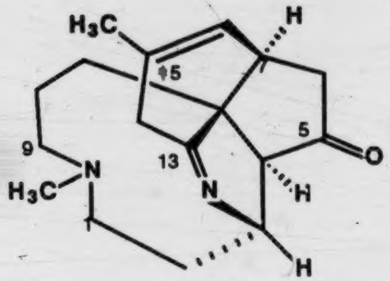
Analysis of 1D and 2D ^{1}H nmr spectra shows that the α, β-unsaturated imine in obscurinine (13) is replaced by a β, γ-unsaturated imine in iso-obscurinine (14). The olefinic hydrogen (δ6.26) in the ^{1}H nmr spectrum of obscurinine (**13**) is replaced by a methylene group, which appears as an AB quartet (δ3.00 and 2.92) in the ^{1}H nmr spectrum of iso-obscurinine (**14**) (see table 3). The 2D COSY 90 spectrum of iso-obscurinine (**14**) (figure 8) reveals two spin systems I and II (scheme 3). Spin system II is consistent with partial structure B, which is also present in obscurinine (**13**). The correlations of spin system I indicate that the AB quartet (δ3.00 and 2.92) is coupled to an olefinic hydrogen (δ5.54) as well as to an alkenic methyl group (δ1.78). The olefinic hydrogen is also coupled to a methine hydrogen (δ2.67), which is in turn coupled to methylene hydrogens (δ2.44 and 1.87). The methylene hydrogens are also coupled to a methine hydrogen (δ4.21) and so forth as summarized in spin system I, partial structure A. Partial structures A and B allow structure **14** to be formulated for iso-obscurinine.


H 5.54
H 2.67
1.78 H₃C
H 1.87
3.00,
2.92
H
H
C
H 2.44
O
2.24
2.58 H
H
N
H 3.81
C
N
H 4.21
2.21 CH₃
H
H
2.08-1.85

(A)

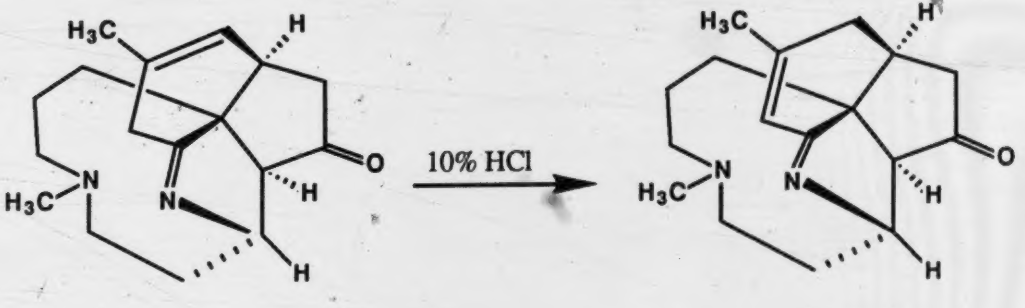

2.27-2.11
2.31 H
H
H
H 1.66-1.50
N
C
H
H
1.66-1.50

(B)



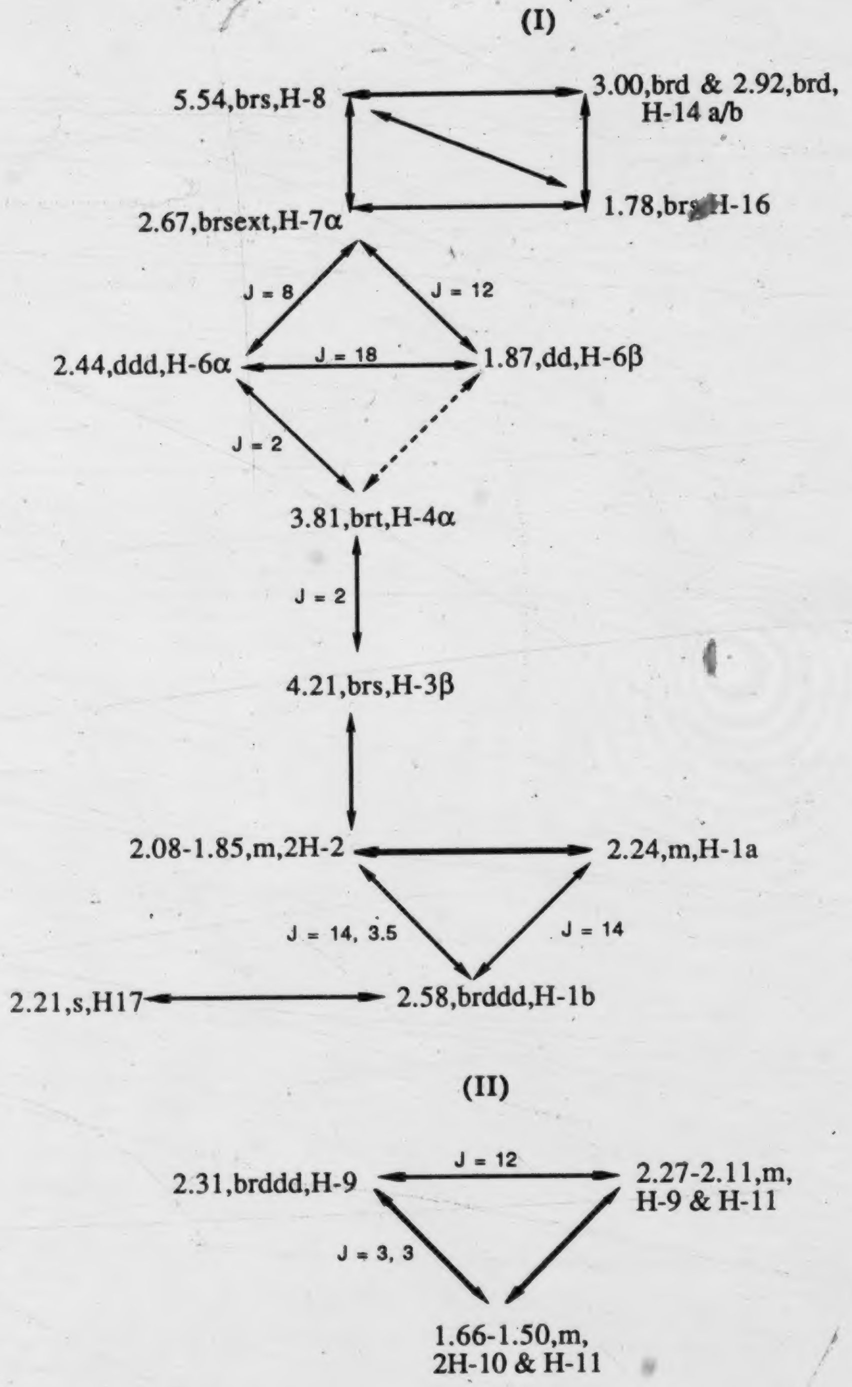
14

The ^{13}C APT experiment (table 4) with iso-obscurinine is consistent with structure 14. The carbon-13 signals are similar to those of obscurinine (13) (see table 4). The major chemical shift differences between obscurinine (13) and iso-obscurinine (14) are at carbons 7, 8, 13, 14, and 15. In the hreims iso-obscurinine (14) shows a fragmentation pattern which resembles that of obscurinine (13). In particular, the low molecular weight ions involving ring A are identical (see experimental). Iso-obscurinine isomerizes to obscurinine when treated with 10% hydrochloric acid, providing further proof for structure of iso-obscurinine (14).



14

13


(I)
5.54,brs,H-8
3.00,brd & 2.92,brd, H-14 a/b
2.67,brsext,H-7α
1.78,brs,H-16
J = 8
J = 12
2.44,ddd,H-6α
J = 18
1.87,dd,H-6β
J = 2
3.81,brt,H-4α
J = 2
4.21,brs,H-3β
2.08-1.85,m,2H-2
2.24,m,H-1a
J = 14, 3.5
J = 14
2.21,s,H17
2.58,brddd,H-1b
(II)
2.31,brddd,H-9
J = 12
2.27-2.11,m, H-9 & H-11
J = 3, 3
1.66-1.50,m, 2H-10 & H-11

Scheme 3. ^{1}H-^{1}H chemical shift correlations obtained from the COSY 90 spectrum of iso-obscurinine (**14**).

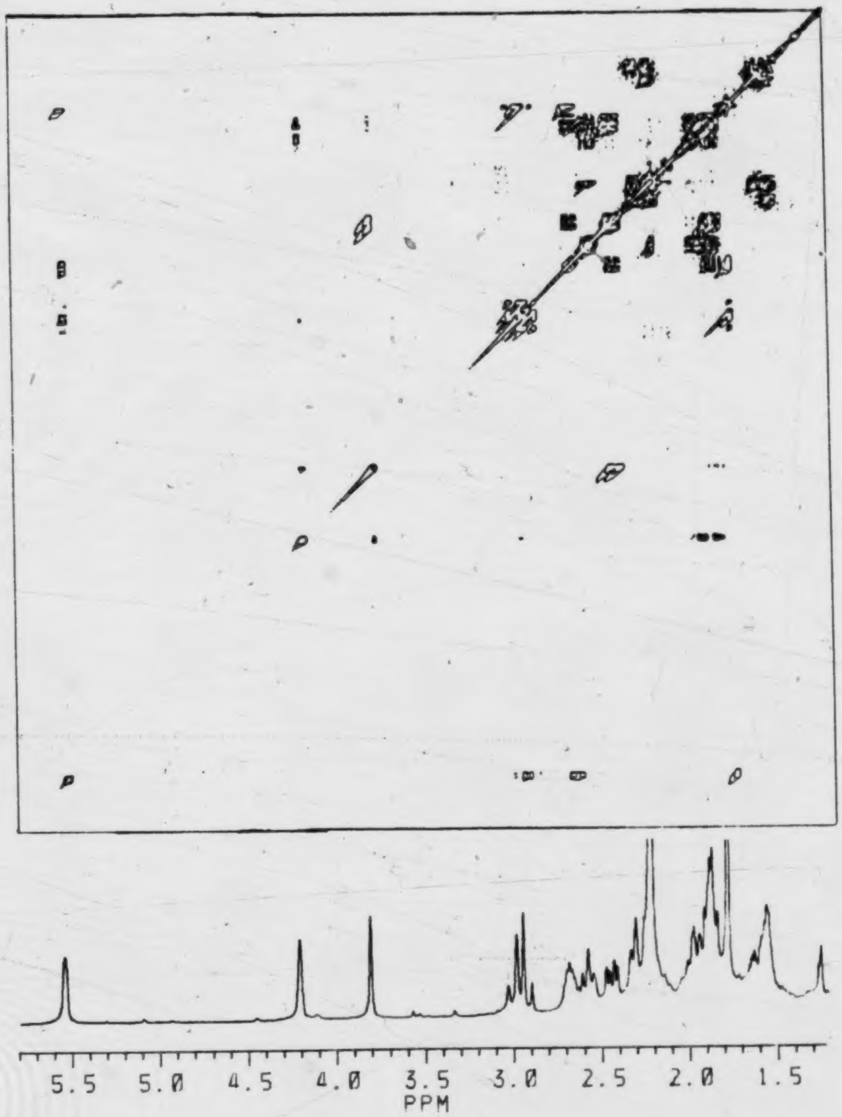
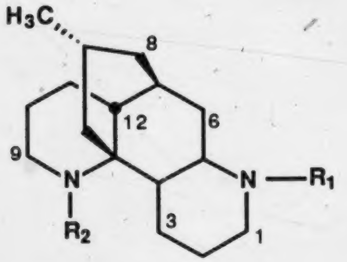
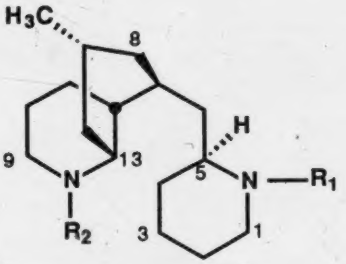


Figure 8. COSY 90 spectrum of iso-obscurinine (14) ($CDCl_3$, 360 MHz).

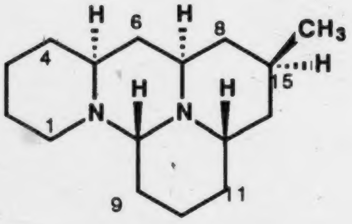
To date, the known dinitrogen Lycopodium alkaloids are of four structural types: flabellidane (**15**), phlegmarane (**16**), cernuane (**17**), and base R (**18**). Base R is the only member of its class. A summary of the proposed biogenesis of the first three skeletons is as shown in scheme 4[3c]. Lysine (**19**) and acetate (**20**) are the fundamental building blocks, which give rise to pelletierine (**21**) and intermediate **22**, from which the flabellidane, phlegmarane and cernuane Lycopodium alkaloids may be formed. A possible biogenetic route to other Lycopodium ring systems, including lucidulane (23), lycopodane (24), fawcettimane (25), fawcettidane (26), and related skeletons is shown in scheme 5[3c].


H_3C
8
12
6
9
N
N —R_1
R_2
3
1

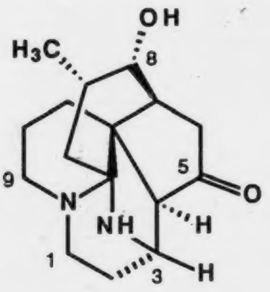
15


H_3C
8
H
9
13
5
N
N —R_1
R_2
3
1

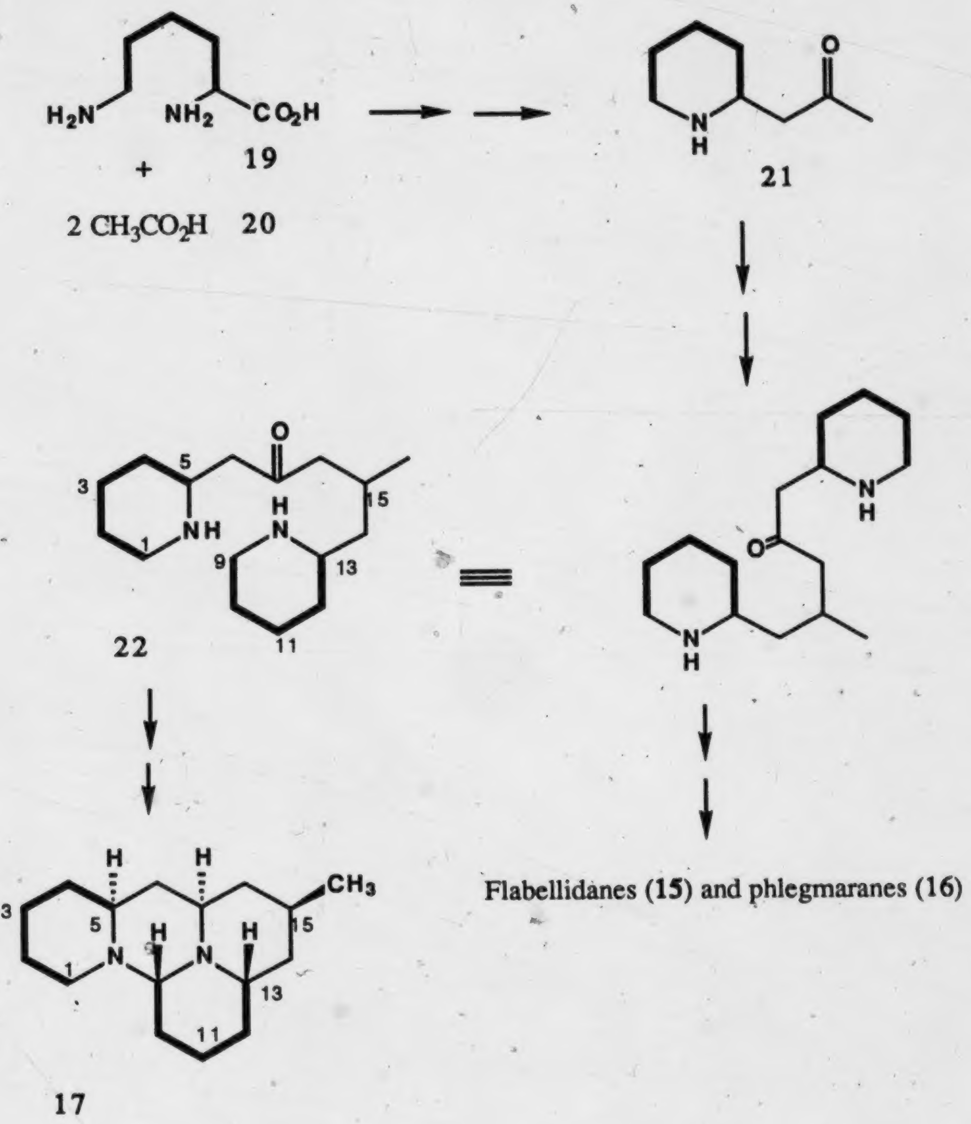
16


H
6
H
8
CH_3
4
H
15
N
H
N
H
1
9
11

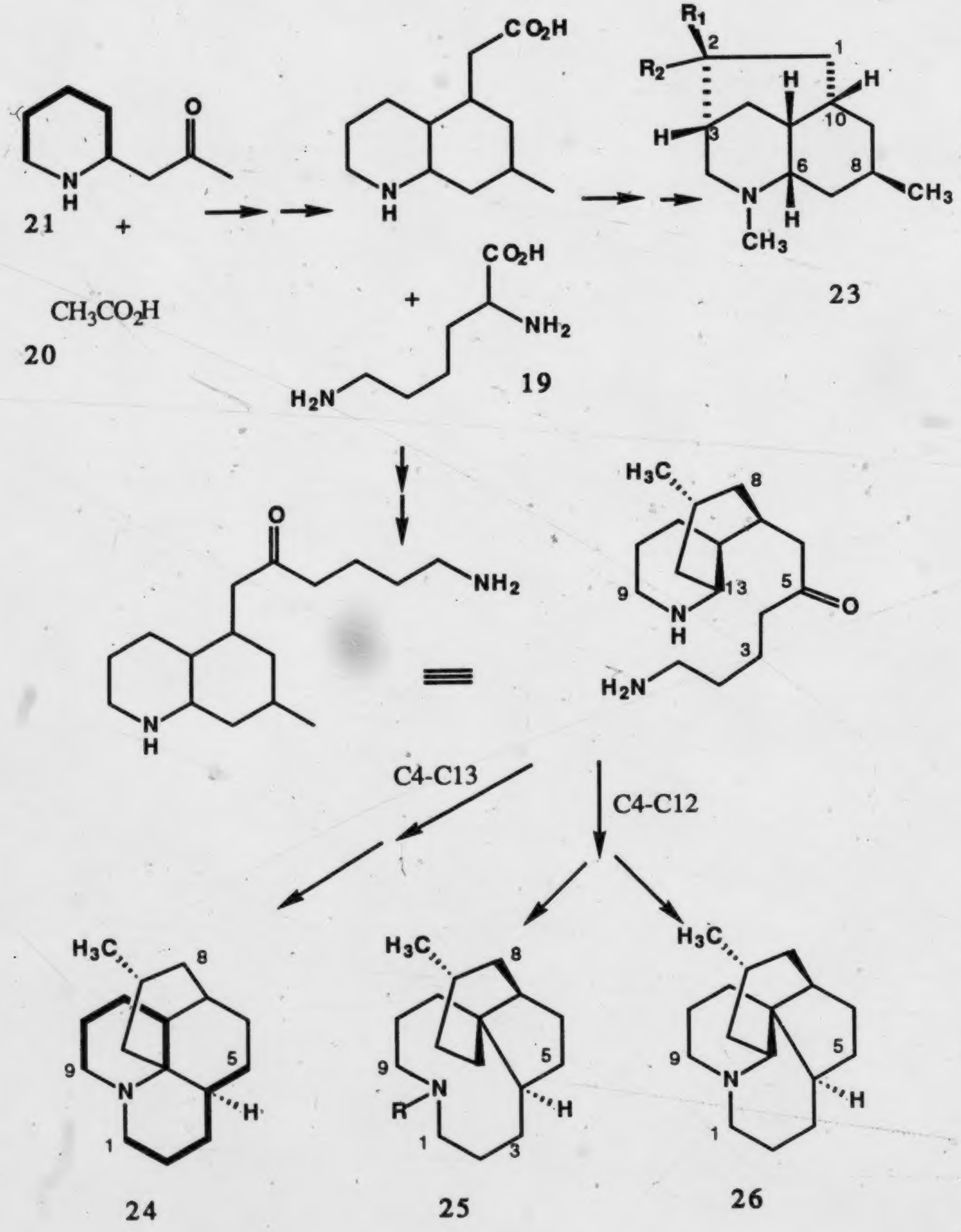
17


OH
H_3C
8
9
5
O
N
NH
H
1
3
H

18


H₂N
NH₂
CO₂H
19
+
2 CH₃CO₂H 20
O
N
H
21
22
3
5
1
NH
O
H
N
9
13
11
15
≡
17
H
H
H
H
CH₃
3
5
1
N
N
13
11
15
Flabellidanes (15) and phlegmaranes (16)

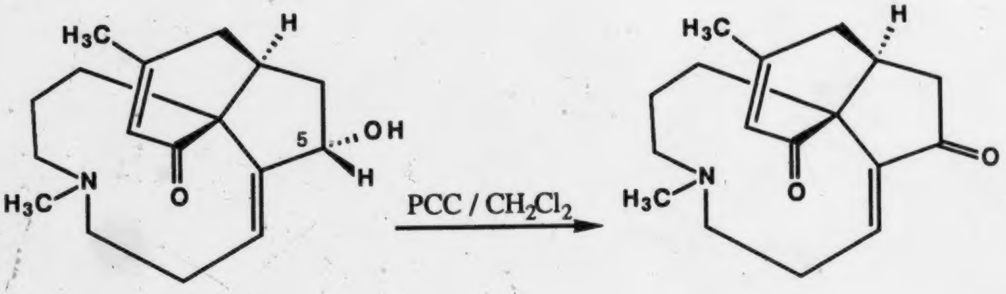
Scheme 4. Summary of the biogenesis of flabellidane (15), phlegmarane (16), and cernuane (17) Lycopodium alkaloid skeletons.


21
20
CH3CO2H
19
23
24
25
26
C4-C13
C4-C12

Scheme 5. Summary of the biogenesis of lucidulane (**23**), lycopodane (**24**), fawcettimane (**25**), fawcettidane (**26**), and related Lycopodium alkaloid skeletons.

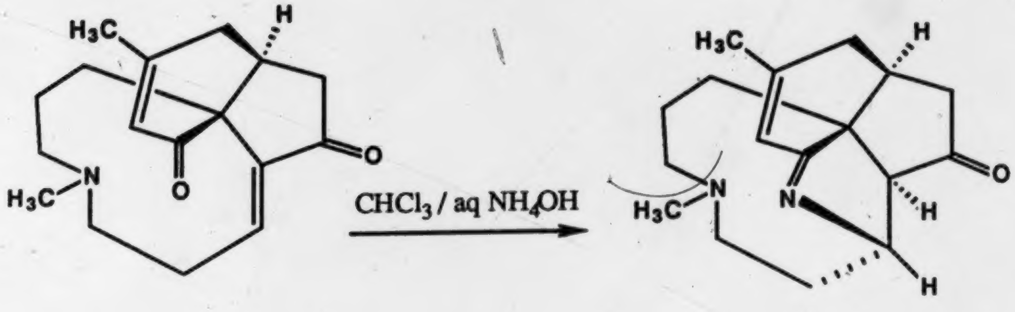
None of these pathways account for the presence of a nitrogen atom between carbons 3 and 13 as observed in obscurinine (**13**), iso-obscurinine (**14**), and base R (**18**). We felt that obscurinine and iso-obscurinine might be artefacts derived by addition of ammonia to an appropriate fawcettimane (**25**) class precursor during the isolation process (ammonia was used to neutralize the aqueous acid used in the isolation). A similar biogenetic pathway was evisioned for the formation of base R by MacLean[3c].

As proof for this hypothesis, lobscurinol (**27**) (structure *vide infra*) was oxidised to lobscurinine (**28**). Upon overnight exposure to aqueous ammonia at room temperature, lobscurinine (**28**) afforded obscurinine (**13**). Obscurinine prepared from lobscurinine is indistinguishable from that isolated earlier (tlc behavior, hreims, and ^{1}H nmr spectrum). At the present time we are awaiting additional plant material in order that we may repeat the extraction avoiding use of ammonia. We anticipate that lobscurinine (**28**) will be obtained from the plant.



27

28



28 13

Lobscurinol (27) epilobscurinol (29) and acetyllobscurinol (30).

The isolation of three closely related bases which we have named lobscurinol (27), epilobscurinol (29) and acetyllobscurinol (30), provide support for the hypothesis that obscurinine (13) and iso-obscurinine (14) are artefacts. As the names suggest, lobscurinol (27) and epilobscurinol (29) are epimers, while acetyllobscurinol (30) is the acetyl derivative of lobscurinol (27). Bases 27 and 29 were isolated from the more polar fractions, whereas base 30 was isolated from the least polar fractions. Bases 27, 29, and 30 are readily detected on chromatograms of crude mixtures by their uv absorption at 254 nm.

The isolation of lobscurinol (27), the most abundant of the three bases, was complicated by the co-occurrence of large amounts of β-lofoline (7c) and other minor bases of similar polarity. Relatively pure lobscurinol was obtained as an opaque solid after several column and preparative thin-layer chromatograms. More pure lobscurinol was obtained in the form of its acetyl derivative 30.

The hreims of lobscurinol (27) indicates a molecular formula of $C_{17}H_{25}NO_2$ (275.1881). The oxygen atoms in the formula are present as a ketone carbonyl (ftir:

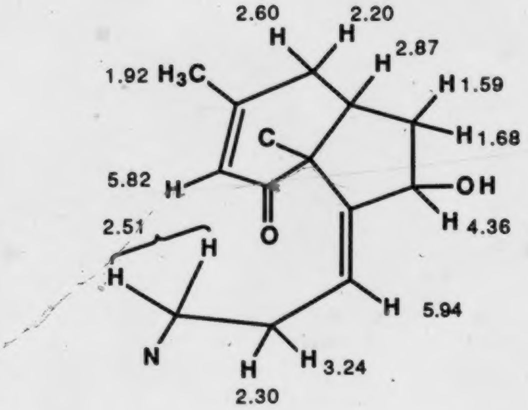
1658 cm^{-1}; ^{13}C nmr: δ200.2 (s)) and hydroxyl (ftir: 3600-3200 cm^{-1}; ^{13}C nmr: δ72.2(d)) groups. In addition to the carbonyl group, four other sp^2 hybridized carbons are present in the ^{13}C nmr spectrum: δ156.4 (s), 141.7 (s), 135.5 (d), and 124.8 (d). The uv spectrum of acetyllobscurinol (**30**) λ_{max} (log ε): 234 (3.3) nm suggests the presence of an α, β - unsaturated cyclohexanone. This information and the fact that the molecular formula of lobscurinol indicates six sites of unsaturation (a carbonyl group and two double bonds) allows us to conclude that lobscurinol (**27**) is tricyclic.

The 1D ^{1}H nmr spectrum of lobscurinol (table 7) shows two olefinic hydrogens (δ5.95 and 5.82), a carbinyl hydrogen (δ4.36), and two methyl groups (δ2.33 and 1.92, for a methyl group attached to nitrogen and a methyl group on an sp^2 hybridized carbon, respectively).

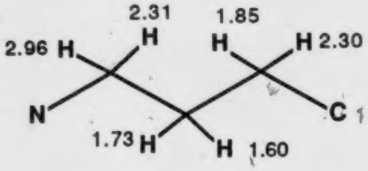
Analysis of the COSY 90 ^{1}H nmr spectrum of lobscurinol (figure 9) reveals two isolated spin systems I and II (scheme 6). Spin system II is consistent with three contiguous methylenes as shown in partial structure B. This partial structure is also found in obscurinine (**13**) and iso-obscurinine (**14**). Correlations in spin system I show that the olefinic hydrogen (δ5.82) is coupled to an alkenic methyl (δ1.92) and methylene group (δ2.60 and 2.20). The methylene group is also coupled to a methine hydrogen (δ2.87), which is further coupled to a methylene group (δ1.68 and 1.59). The methylene hydrogen (δ1.59) is coupled to a carbinyl hydrogen (δ4.36), which is in turn coupled to the olefinic hydrogen (δ5.94). The olefinic hydrogen (δ5.49) is further coupled to an allylic methylene group (*ca* δ2.56-2.51). Partial structure A is consistent with this analysis.

The APT experiment (table 8) shows the seventeen carbons in the formula (11 quaternary / CH$_2$ signals and 6 CH$_3$ / CH signals). Five of the six CH$_3$ / CH signals are due to olefinic CH's (δ132.0 (d) and 124.8 (d)), one carbinyl CH (δ74.2), and two methyl groups (δ48.3 (q) and 24.3 (q)). The remaining CH signal (δ38.4) is correlated to the only methine hydrogen resonating at δ2.87 in the ^{1}H nmr spectrum of lobscurinol.

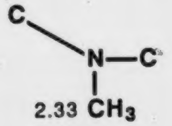
An APT experiment, in which the variable delay is half the delay of a normal APT experiment, reveals that four of the eleven quaternary / CH_2 signals are due to quaternary carbons (δ 200.2, 157.1, 146.9, and 58.3). Therefore, there are seven methylene groups in the molecule. Partial structures A, B, C are consistent with the results to this point and allow structure **27** to be formulated for lobscurinol.


2.60
2.20
H
H
2.87
H
1.92 H₃C
H 1.59
H 1.68
C
5.82 H
OH
H 4.36
2.51
H
O
H
H 5.94
N
H
H 3.24
2.30

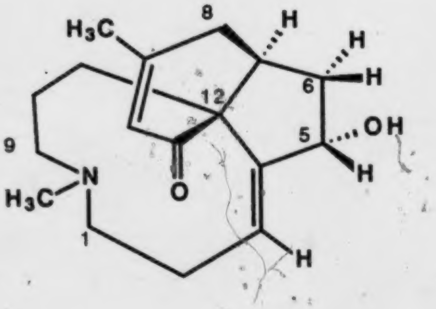
(A)


2.31
1.85
2.96 H
H
H
H 2.30
N
C
1.73 H
H 1.60

(B)



(C)


H₃C
8
H
H
H
6
12
5
OH
9
H
N
H₃C
O
1
H

27

Table 7 ^{1}H nmr spectra of lobscurinol (27), acetyllobscurinol (30), and acetylepilobscurinol (31) ($CDCl_3$, 400 MHz).

		Chemical shift in δ, mult., J in Hz.			
H	27	H	30	H	31
3	5.94,ddd,11.5,6.5,1	3	6.02,brddd,11.5,6.5,1	14	5.85,brs,$W_{1/2}$=5
14	5.82,brs,$W_{1/2}$=5	14	5.82,brs,$W_{1/2}$=5	3	5.79,ddd,11.5,7.0,2.0
5β	4.36,dd,5,1	5β	5.40,brd,5	5α	5.45,dddd,7,7,3,2
2a	3.24,dddd,19,11.5,11.5,4	2a	3.37,dddd,17,11.5,11.5,2	2a	3.25,dddd,17,11.5,11.5,2
9a	2.96,ddd,14,11.5,3	9a	3.09,brddd,14,11.5,4	9a	2.98,brdd,13.5,13.5
7α	2.87,brddd,13,6,6	7α	2.87,brddd,11.5,7,7		
8α	2.60,brdd,19,6	8α & 1a/b	2.68-2.56,m		
2a/b	2.56-2.51,m				
17(3H)	2.33,s	17(3H)	2.35,s	17(3H)	2.34,s
2a,9b &11a	2.31-2.25,m	(4H)	2.42-2.20,m		
8β	2.20,brd,19	8β	2.18,brd,20		
16(3H)	1.92,brs	16(3H)	1.96,brs	16(3H)	1.92,s
11a	1.85,ddd,15,8,2	(1H)	2.09-2.02		
10a	1.73,m	(3H)	1.71-1.50		
6α	1.68,dd,13,6				
10b	1.60,m				
6β	1.59,ddd,13,13,5				
		$COC\underline{H}_3$	2.02,s	$COC\underline{H}_3$	2.02,s

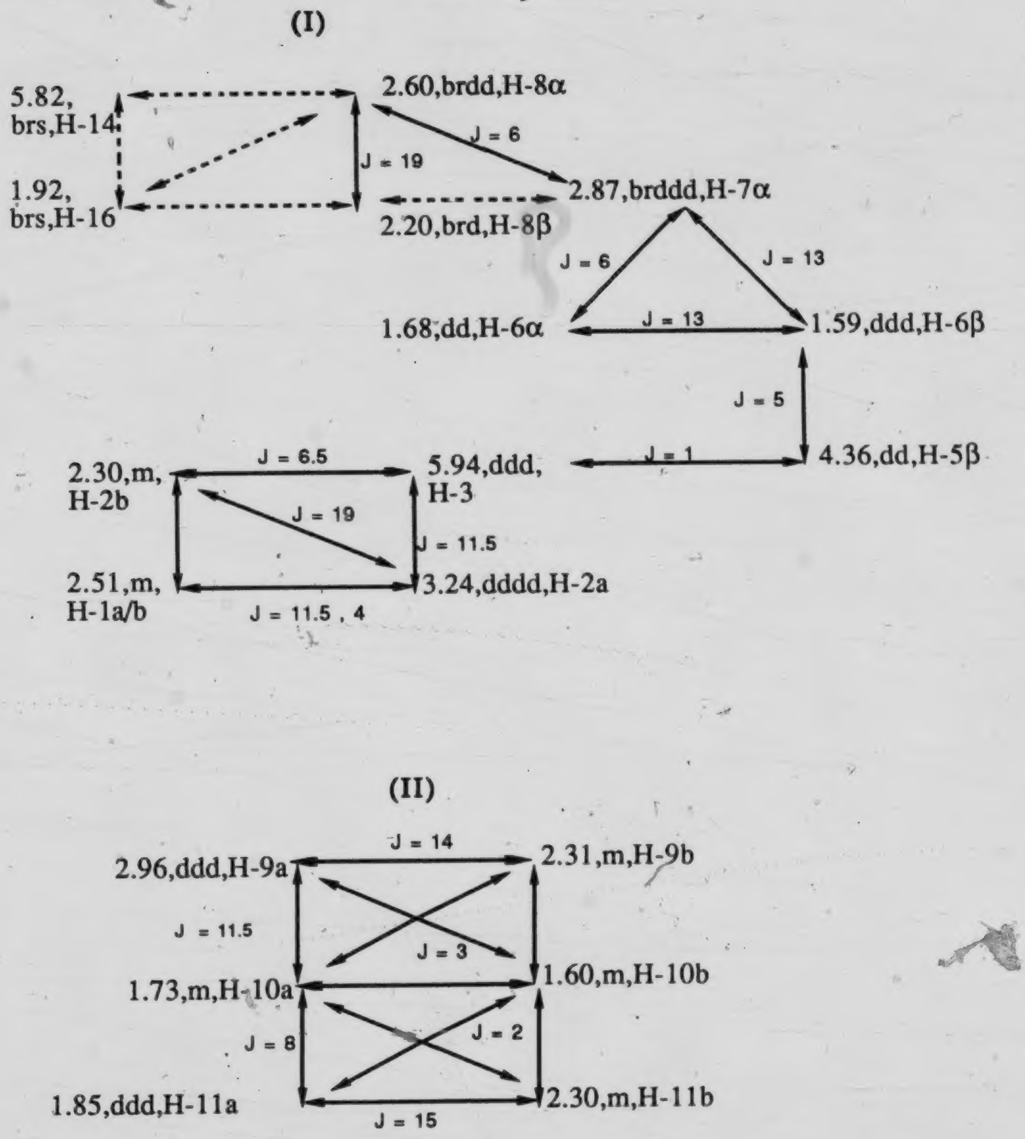
Table 8 ^{13}C nmr spectra of lobscurinol (27), acetyllobscurinol (30), and lobscurinine (28) ($CDCl_3$,100 MHz).

		Chemical shift in δ.		
C	mult.	27	30	28[c]
13	s	200.2	199.8	198.7
15	s	157.1	156.4	156.9
4	s	146.9	141.7	139.7
3	d	132.0	135.5	142.0
14	d	124.8	124.8	125.0
5	d	74.2	77.1	202.9(s)
12	s	58.3	57.5	57.1
9	t	57.0[a]	56.1	54.4[a]
1	t	56.3[a]	53.9	54.7[a]
17	q	48.3	47.7	47.6
7	d	38.4	38.1	35.2
6	t	37.3	35.5	41.3
8	t	30.3[b]	30.5[a]	29.8[b]
11	t	30.0[b]	29.8[a]	28.9[b]
2	t	29.9[b]	28.5[a]	28.9[b]
10	t	25.6	25.5	25.5
16	q	24.3	24.2	24.5
			170.6(s,$\underline{C}OCH_3$)	
			21.5(q,$CO\underline{CH_3}$)	

a, signals may be reversed.

b, " " " "

c, spectrum obtained at 70.5 MHz


(I)
5.82, brs,H-14
2.60,brdd,H-8α
J = 6
J = 19
1.92, brs,H-16
2.87,brddd,H-7α
2.20,brd,H-8β
J = 6
J = 13
1.68,dd,H-6α
J = 13
1.59,ddd,H-6β
J = 5
J = 6.5
2.30,m, H-2b
5.94,ddd, H-3
J = 1
4.36,dd,H-5β
J = 19
J = 11.5
2.51,m, H-1a/b
3.24,dddd,H-2a
J = 11.5 , 4
(II)
J = 14
2.96,ddd,H-9a
2.31,m,H-9b
J = 11.5
J = 3
1.73,m,H-10a
1.60,m,H-10b
J = 8
J = 2
1.85,ddd,H-11a
2.30,m,H-11b
J = 15

Scheme 6. ^1H-^1H chemical shift correlations obtained from the COSY 90 spectrum of lobscurinol (**27**).

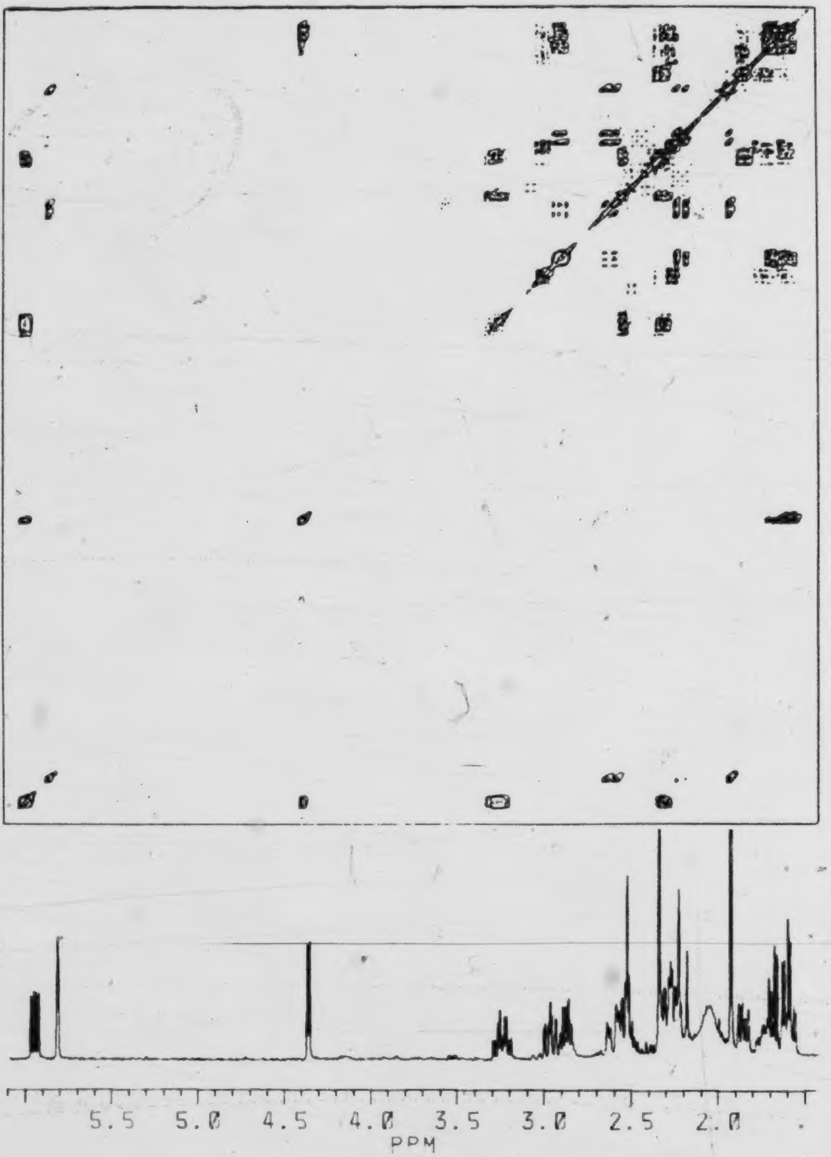


Figure 9. COSY 90 spectrum of lobscurinol (27) ($CDCl_3$, 360 MHz).

The relative stereochemistry at C-5 and C-7 of lobscurinol **(27)** is established on the basis of extensive decoupling of ^{1}H nmr spectra (table 9) and by nOe difference spectroscopy (table 10). The magnitude of $J_{7,8\beta}$ (*ca* 0 Hz) and $J_{7,8\alpha}$ (6 Hz) indicates that H-7 is pseudo *axial* and α-oriented. Upon irradiation of H-7 (δ 2.87, ddd, J = 13,6,6 Hz), one H-6 (δ 1.68, dd, J = 13,6 Hz) collapses to doublet (J = 13 Hz), while its *geminal* partner (δ 1.59, ddd, J = 13,13,5 Hz) is perturbed*. Since H-7 is pseudo *axial* and α-oriented, then the hydrogen at δ 1.59 must be β-oriented, and *antiperiplanar* to H-7 ($J_{7\alpha,6\beta}$ = 13 Hz). The carbinyl hydrogen (δ 4.36, dd, J = 5,1 Hz) is β-oriented and *cis* to H-6β (δ 1.59). Upon irradiation of H-5 (δ 4.36, dd, J = 5,1 Hz), H-3 (δ 5.94, ddd, J = 11.5,6.5,1 Hz) collapses to double doublet (J = 11.5,6.5 Hz) and H-6β (δ 1.59, ddd, J = 13,13,5 Hz) collapses to double doublet (J = 13,13 Hz). Furthermore, both H-3 and H-6β are enhanced upon irradiation of H-5 in the nOe experiment. As well, the signal for the hydrogen at C-11 (δ 1.85) is enhanced on irradiation of H-7 indicating that H-7 is *cis* to the C-11 to C-12 bond, providing further proof that H-7 is α-oriented.

In the hreims of lobscurinol **(27)** there is a base peak at m/z 84, $C_5H_{10}N$, which may arise from fragmentation of ring A. A fragmentation scheme for this ion as well as other abundant fragment ions is shown in scheme 7. A similar fragmentation pattern has been proposed to account for the fragment ions observed in the mass spectra of magellanine **(32)**[25] and paniculatine **(33)**[26].

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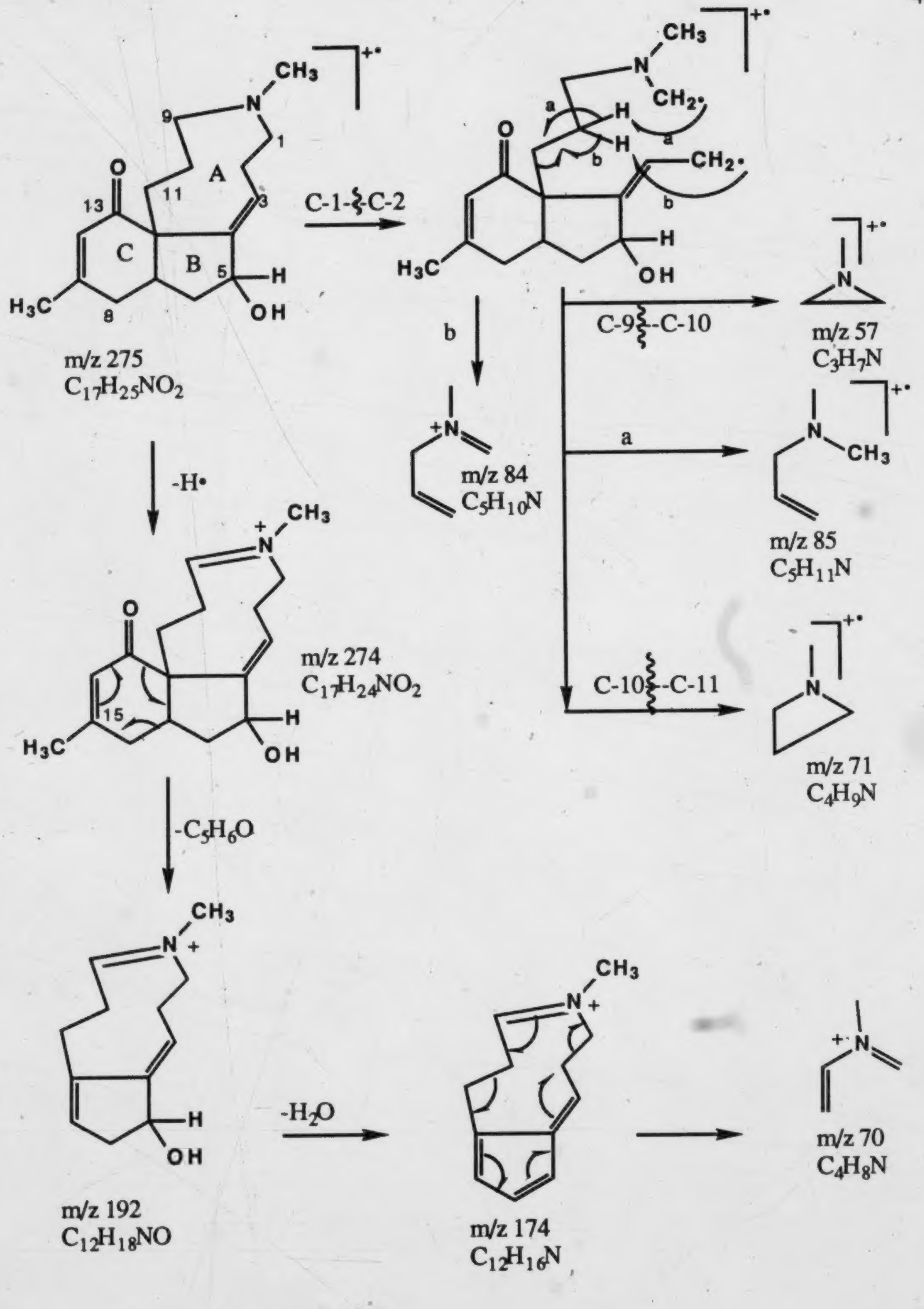
* Signal partially obscured by overlapping signals.

Table 9 Decoupling experiments in the ^{1}H nmr spectrum of lobscurinol (27) ($CDCl_3$, 400 MHz).

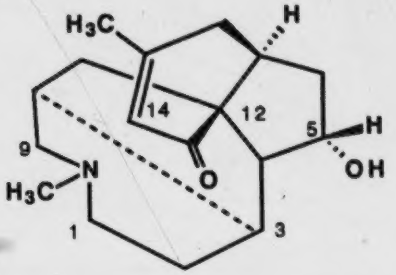
Signal irradiated	Chemical shift in δ, mult., J in Hz.	Observed change
5.94,ddd,11.5,6.5,1(H-3)	4.36,dd,5,1(H-5β)	d,5
	3.24,dddd,19,11.5,11.5,4(H-2)	ddd,19,11.5,4
	2.31-2.25,m(H-2)	perturbed(pert.)
5.82,brs,$W_{1/2}$=2(H-14))	2.60,brdd,19,6(H-8α)	sharper,brdd,19,6
	2.20,brd,19(H-8β)	sharper,brd,19
4.36,dd,5,1(H-5β)	5.94,ddd,11.5,6.5,1(H-3)	dd,11.5,6.5
	1.59,ddd,13,13,5(H-6β)	dd,13,13
3.24,dddd,19,11.5,11.5,4(H-2)	5.94,ddd,11.5,1(H-3)	J=11.5,vanished
	2.56-2.51,m(H-1a/b)	pert.
	2.31-2.23,m(H-2a/b)	pert.
2.96,ddd,14,11.5,3(H-9a)	2.31-2.25,m(H-9b)	pert.
	1.73,m(H-10a)	pert.
	1.63,m(H-10b)	pert.
2.87,brddd,13,6,6(H-7α)	2.60,brdd,19,6(H-8α)	brd,19
	1.68,dd,13,6(H-6α)	d,13
	1.59,ddd,13,13,5(H-6β)	pert.
1.85,ddd,15,8,2(H-11a)	2.31-2.25,m(H-11b)	pert.
	1.73-1.60,m(H-10a/b)	pert.
1.73,m(H-10a & 6α)	2.96,ddd,14,11.5,3(H-9a)	pert.
	2.87,brddd,13,6,6(H-7α)	pert.
	2.31-2.25,m(H-9b)	pert.
	1.85,ddd,15,8,2(H-11a)	pert.
	1.59,ddd,13,13,5(H-6β)	pert.
1.59,m(H-10b & 6β)	2.96,ddd,14,11.5,3(H-9a)	J=3,vanished
	2.87,brddd,13,6,6(H-7α)	pert.
	2.31-2.25,m	pert
	1.85,ddd,15,8,2(H-11a)	dd,15,8
	1.73,m(H-10a & 6α)	pert.

Table 10 ^1H nmr nOe experiments with lobscurinol **(27)** ($CDCl_3$, 400 MHz).

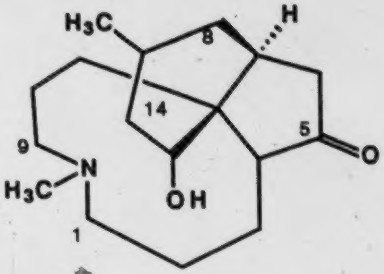
	Chemical shift in δ.
Signal saturated	**Signal enhanced (% nOe)**
5.49(H-3)	4.36(H-5)(6.7)
	3.24(H-2a)(3.4)
	2.30(H-2b)(6.7)
5.82(H-14)	1.92(H-16)(1.2)
4.36(H-5)	5.94(H-3)(8.2)
	1.59(H-6β)(9.2)
3.24(H-2a)	5.94(H-3)(1.2)
	2.30(H-2b)(16.7)
2.96(H-9a)	2.30(H-9b)(16.0)
2.87(H-7α)	1.85(H-11a)(6.2)
1.92(H-16)	5.82(H-14)(10.0)
1.85(H-11a)	2.31(H-11b)(17.6)


CH3
N
+•
9
1
O
13
11
A
3
C
B
5
H
H3C
8
OH
m/z 275
C17H25NO2
C-1-C-2
a
b
CH2•
-H•
b
m/z 84
C5H10N
C-9-C-10
m/z 57
C3H7N
m/z 85
C5H11N
C-10-C-11
m/z 71
C4H9N
m/z 274
C17H24NO2
15
-C5H6O
m/z 192
C12H18NO
-H2O
m/z 174
C12H16N
m/z 70
C4H8N

Scheme 7. Fragmentation in the hreims of lobscurinol (27).



32



33

Acetyllobscurinol (**30**), was isolated as a colourless opaque solid, $[\alpha]_D$ +110°. A molecular weight of 317.1985 ($C_{19}H_{27}NO_3$) was indicated by its hreims and confirmed by cims. In the ftir, base **30** shows the absence of hydroxyl group(s), but indicates the presence of an acetoxyl group (1735 and 1240 cm^{-1}) and an α, β-unsaturated carbonyl (1659 cm^{-1}). The uv spectrum supports the presence of an α, β-unsaturated ketone, λ_{max} ($\log \varepsilon$): 234 (3.3) nm.

The ^1H and ^{13}C nmr spectra (tables 7 and 8, respectively) are very similar to those of lobscurinol (**27**). In the ^1H nmr spectrum, the absorption at δ 5.40 (brd, J = 3 Hz) is due to a carbinyl hydrogen *geminal* to an acetoxyl group. The presence of an acetoxyl group is confirmed by signals in the ^1H nmr (δ 2.02, s, 3H) and ^{13}C nmr (δ 170.6 (s) and 21.5 (q)) spectra.

The relative stereochemistry for acetylloboscurinol (**30**) is shown to be the same as that for lobscurinol (**27**). Upon acetylation (Ac_2O, Et_3N) lobscurinol (**27**) afforded a monoacetyl derivative (**30**), which is identical with the naturally occurring compound in all respects including the optical rotation. The relative and absolute stereochemistry can be correlated with that of lycoplegmarine (**34**)[27]. In the ^1H nmr, lycoplegmarine (**34**) and acetyllycoplegmarine (**35**) show signals which are the same as those of lobscurinol (**27**) and acetyllobscurinol (**30**) (see table 11 for comparison). The relative and absolute stereochemistry of lycoplegmarine (**34**) was established by X-ray crystallography and application of the Brewster's benzoate rule to a suitable derivative[28].

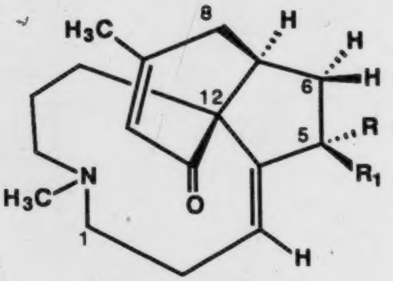
Table 11 Comparison of some ^1H nmr spectral data of lobscurinol (**27**) and acetyllobscurinol (**30**) with that of lycoplegmarine (**34**) and acetyllycoplegmarine (**35**).

	Chemical shift in δ, mult., J in Hz.			
H	27[a]	34[b]	30[a]	35[b]
H-16	1.92, s	1.88, s		
NCH_3	2.33, s	2.32, s		
H-5	4.36, dd, 5.0,1.0	4.36, brs, $W_{1/2} = 7$	5.40, brd,3	5.40, brs, 7
H-3	5.94, ddd, 11.5,6.5,1.0	5.94, dd, 12.0,6.0		

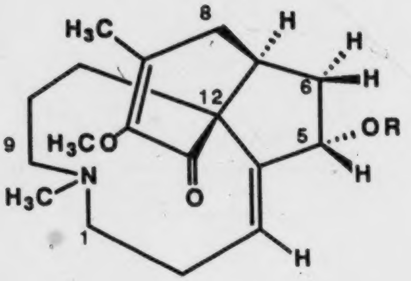
a, spectra obtained at 400 MHz.

b, spectra obtained at 100 MHz.

Base **29** was identified as the C-5 epimer of lobscurinol (**27**) by comparison of spectral data (hreims and ^1H nmr) with that of lobscurinol (**27**) and acetyllobscurinol (**30**). The name epilobscurinol was therefore assigned to base **29**. Epilobscurinol (**29**) occurs in *L. obscurum* in much smaller quantity than lobscurinol (**27**). It has a lower Rf value than lobscurinol (**27**) and is eluted after lobscurinol when the bases are separated by column chromatography. Epilobscurinol (**29**) was purified and identified as its acetyl derivative **31**.



27 R = OH, R_1 = H
29 R = H, R_1 = OH
30 R = OAc, R_1 = H
31 R = H, R_1 = OAc



34 R = H
35 R = AC

In its hreims acetylepilobscurinol (**31**) shows a molecular formula of $C_{19}H_{27}NO_3$ (317.1985) and a fragmentation pattern which resembles that of lobscurinol (**27**) and acetyllobscurinol (**30**). The base peak at m/z 84 ($C_5H_{10}N$) is also the base peak in the hreims of lobscurinol (**27**).

The ^{1}H nmr spectra of acetylepilobscurinol (**31**) and acetyllobscurinol (**30**) (see table 7) are remarkably similar, except for slight chemical shift differences at C-5 and in the vicinity of C-5. The hydrogen at C-5 in acetylepilobscurinol (**31**) absorbs at δ5.45 (dddd, J = 7, 7, 3, 2 Hz) more downfield than the hydrogen at C-5 in acetyllobscurinol (**30**) (δ5.4, brd, J = 3 Hz). This is consistent with an α-oriented hydrogen at C-5. A hydrogen α-oriented at C-5 is in accord with the coupling pattern observed. H-5α shows *vicinal* coupling to C-6 (J =7 Hz) methylene hydrogens, allylic coupling to a C-3 (J = 2 Hz) olefinic hydrogen, as well as coupling to a C-7 (J = 3 Hz) hydrogen. In acetyllobscurinol (30) H-5β (δ5.40, brd, J = 3 Hz) couples only with H-3. No other spectral data was obtained for acetylepilobscurinol (**31**) due to the limited availability of the sample.

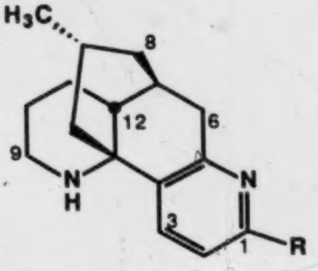
Hydroxypropyllycodine (36).

Base **36**, $C_{19}H_{28}N_2O$ (300.2199) was isolated as a colourless oil. It is optically active ($[\alpha]_D = +11^o$) and absorbs uv light (λ_{max} 272 (3.7) and *ca* 280 (shoulder) nm). The molecular formula was obtained by hreims and cims confirmed the molecular weight.

In the ftir spectrum, a broad peak centered at 3320 cm^{-1} denotes the presence of an NH and / or OH group. The presence of a pyridine ring is suggested by the uv spectrum and by absorption at 1650 (weak), 1590, and 1570 cm^{-1} in the infrared. The ^{13}C nmr spectrum shows that the pyridine ring is trisubstituted (δ 157.6 (s), 157.3 (s), 134.0 (d), 133.7 (s), and 121.4 (d)).

The ^{1}H nmr spectrum of **36** shows two aromatic *ortho* hydrogens (δ 7.71 and 6.96, d, J = 8 Hz), a low field signal at δ 4.21 indicative of a hydrogen *geminal* to an oxygen atom, and two secondary methyl groups (δ 1.27 and 0.78). These data and seven sites of unsaturation in the molecular formula indicate that base **36** possesses a tetracyclic ring skeleton. The fragmentation pattern in the hreims of **36** shows that the majority of fragment ions retain both nitrogens. This suggests that the nitrogens are present as part of the ring skeleton and not as substituent (s).

Careful inspection in the ^{1}H and ^{13}C nmr spectra (tables 12 and 13, respectively) reveals that base **36** resembles lycodine (**10**) in many respects. Base **36** differs from lycodine by a C_3H_6O unit. This information together with the ^{1}H and ^{13}C nmr data suggest that the C_3H_6O unit is incorporated in base **36** as an isolated 2-hydroxypropyl group. The presence of a 2-hydroxylpropyl group was confirmed by ^{1}H nmr decoupling experiments. Upon irradiation of the hydrogen signal at δ 4.21 (ddq, J = 9,3,6 Hz), the *geminal* methylene hydrogens at δ 2.89 (dd, J = 15,3 Hz) and 2.71 (dd, J = 15,9 Hz) each collapse to a doublet (J = 15 Hz), while the methyl group at δ 1.27 (d, J = 6 Hz) collapses to singlet.



10 R = -H
36 R = $-CH_2CH(OH)CH_3$

Comparison of the ^{13}C nmr spectrum of base **36** with that of lycodine reveals that the hydroxypropyl group is substituted at C-1. In the ^{13}C nmr spectrum, C-1 in lycodine appears as a doublet (δ 147), while in base **36** it appears as a singlet (δ 157.6). This is consistent with the observed coupling (J = 8 Hz) of the aromatic hydrogens, since in pyridines $J_{\alpha\beta}$ is normally *ca* 5 Hz, while $J_{\beta\gamma}$ is 7-9 Hz[29].

The correlations shown in scheme 8, derived from analysis of the COSY 90 spectrum (figure 10), provide proof that the hydroxylpropyl substituent is at C-1 in base **36**. The methyl group at C-19 (δ 1.27) is coupled to the carbinyl hydrogen (δ 4.21), which is in turn coupled to an AB quartet (δ 2.83 and 2.71). The AB quartet shows long range coupling to H-2 (δ 6.96), which is *ortho* to H-3 (δ 7.71). Long range coupling is observed from both H-2 and H-3 to the methylene hydrogens at C-6 (δ 3.09 and 2.66) and these hydrogens are further coupled to H-7 (2.06). The remainder of the correlations, from H-7 through to the methylene groups at C-14 (δ 1.43 and 1.13) and C-9 (δ 2.43 and 2.27), are summarized in scheme 8.

Table 12 ^1H nmr spectra of lycodine (10), hydroxypropyllycodine (36), and diacetylhydroxyllycodine (37) ($CDCl_3$, 400 MHz).

	Chemical shift in δ, mult., J in Hz.		
H	10	36	37
1	8.31,dd,4.7,1.6		
2	7.07,dd,7.8,4.7	6.96,d,8	7.00,d,7.9
3	7.73,dd,7.8,1.6	7.71,d,8	7.49,d,7.9
6ax	3.10,dd,18.7,7.2	3.09,dd,19,7	3.17,brdd,18.8,7
9eq	2.72,dm,13.0	2.77,dm,12	3.60,brdq,14.5,2
6eq	2.65,d,18.7	2.66,d,19	2.65,d,18.8
9ax	2.37,ddd,13,11.6,3.5	2.43,dddd,12,12,3,1	2.63,ddd,14.5,11,4
7eq	2.03,brsext,3.4	2.06,brsext,3	2.13,m
8eq	1.72,brdm,12.5	1.77,brdm,12	1.71,brdm,12.8
10a/b &12	1.60-1.45,m	1.60-1.46,m	1.65-1.55,m(H-10a/b)
14eq	1.40,dm,12	1.43,ddd,12,3.5,2	3.17,brdm,12
8ax	1.28,dddd,12,12,3,1	1.33,dddd,12,12,4,1	1.38-1.25,m(H-8ax, 11a,12)
11a/b &15ax	1.23-1.10,m	1.28-1.17,m	1.18,m(H-15ax)
14ax	1.09,dd,11.4,11.4	1.13,dd,11,11	1.79,dd,13,12
16	0.72,d,6	0.78,d,6	0.82,d,6.4
17a		2.89,dd,15,3	3.03,dd,13.7,8.7
17b		2.71,dd,15,9	2.91,dd,13.7,5.7
18		4.21,ddq,9,3,6	5.27,ddq,7.8,5.7,6.4
19		1.27,d,6	1.27,d,6.3

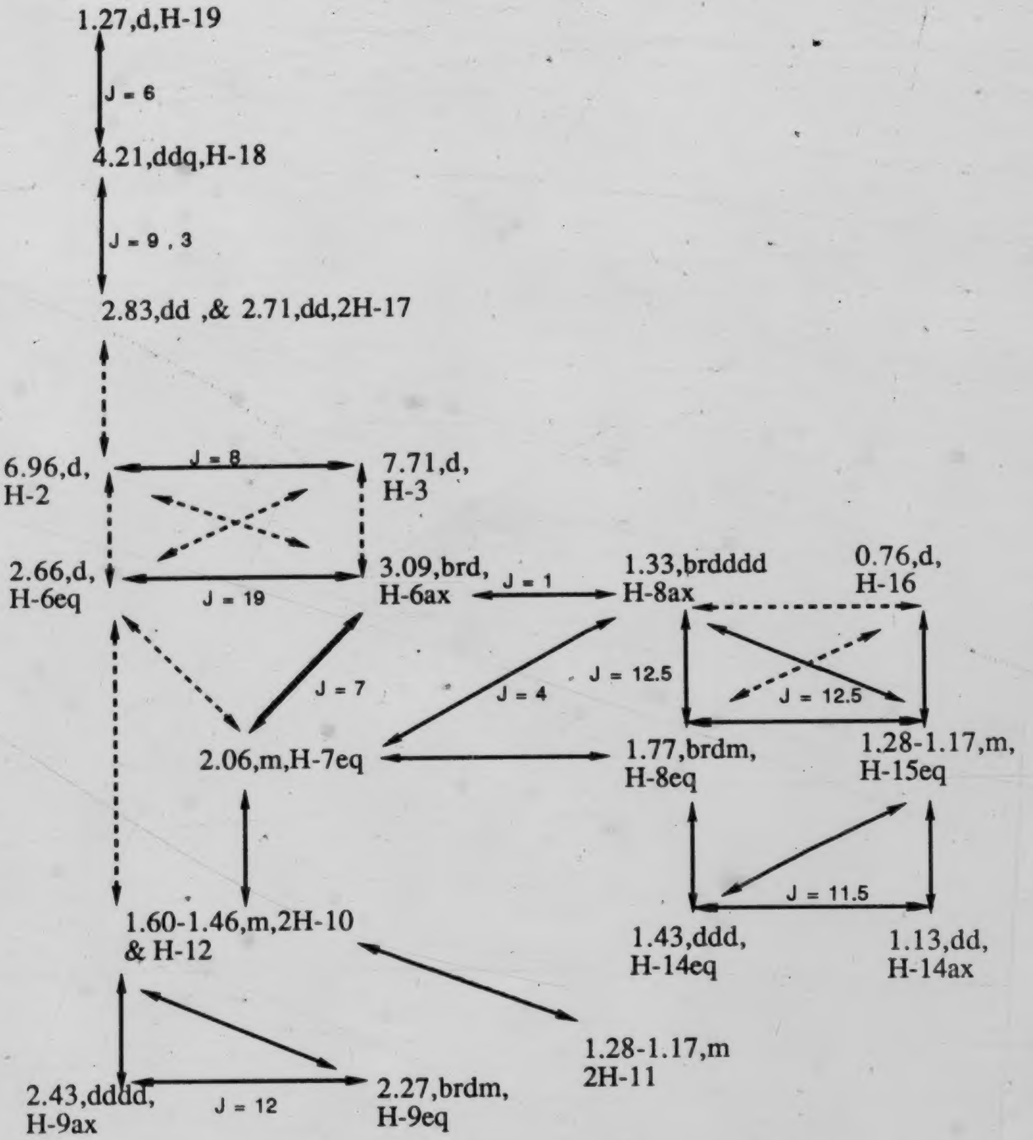
Table 13 ^{13}C nmr spectra of lycodine (**10**), hydroxypropyllycodine (**36**) and diacetylhydroxylpropyllycodine (**37**) ($CDCl_3$, 100.6 MHz).

		Chemical shift in δ.		
C	**mult.**	**10**	**36**	**37**
1	d	147.0	157.6(s)[a]	155.8(s)
2	d	121.6	121.4	121.8
3	d	133.4	134.0	134.8
4	s	136.2	133.7	132.6
5	s	158.9	157.3[a]	157.3
6	t	35.5	35.2	35.0
7	d	34.1	33.8	34.5
8	t	44.0	44.2	44.4
9	t	41.5	41.4	45.6[a]
10	t	26.4	26.2	25.8
11	t	28.2	28.0	27.4
12	d	45.0	44.7	43.9[b]
13	s	56.1	55.9	65.7
14	t	51.7	51.4	47.7[a]
15	d	26.0	25.9	26.5[c]
16	q	22.1	22.1	22.3
17	t		43.7	43.0[b]
18	d		67.1	70.8
19	q		23.1	26.4[c]
$\underline{C}O$	s			172.6
$\underline{C}O$	s			170.4
$\underline{C}H_3CO$	q			21.2
$\underline{C}H_3CO$	q			20.0

a, signals may be reversed.

b, " " " "

c, " " " "


1.27,d,H-19
J = 6
4.21,ddq,H-18
J = 9 , 3
2.83,dd ,& 2.71,dd,2H-17
6.96,d, H-2
J = 8
7.71,d, H-3
2.66,d, H-6eq
J = 19
3.09,brd, H-6ax
J = 1
1.33,brdddd H-8ax
0.76,d, H-16
J = 7
J = 4
J = 12.5
J = 12.5
2.06,m,H-7eq
1.77,brdm, H-8eq
1.28-1.17,m, H-15eq
J = 11.5
1.43,ddd, H-14eq
1.13,dd, H-14ax
1.60-1.46,m,2H-10 & H-12
1.28-1.17,m 2H-11
2.43,dddd, H-9ax
J = 12
2.27,brdm, H-9eq

Scheme 8. ^{1}H-^{1}H chemical shift correlations obtained from the COSY 90 spectrum of hydroxypropyllycodine (**36**).

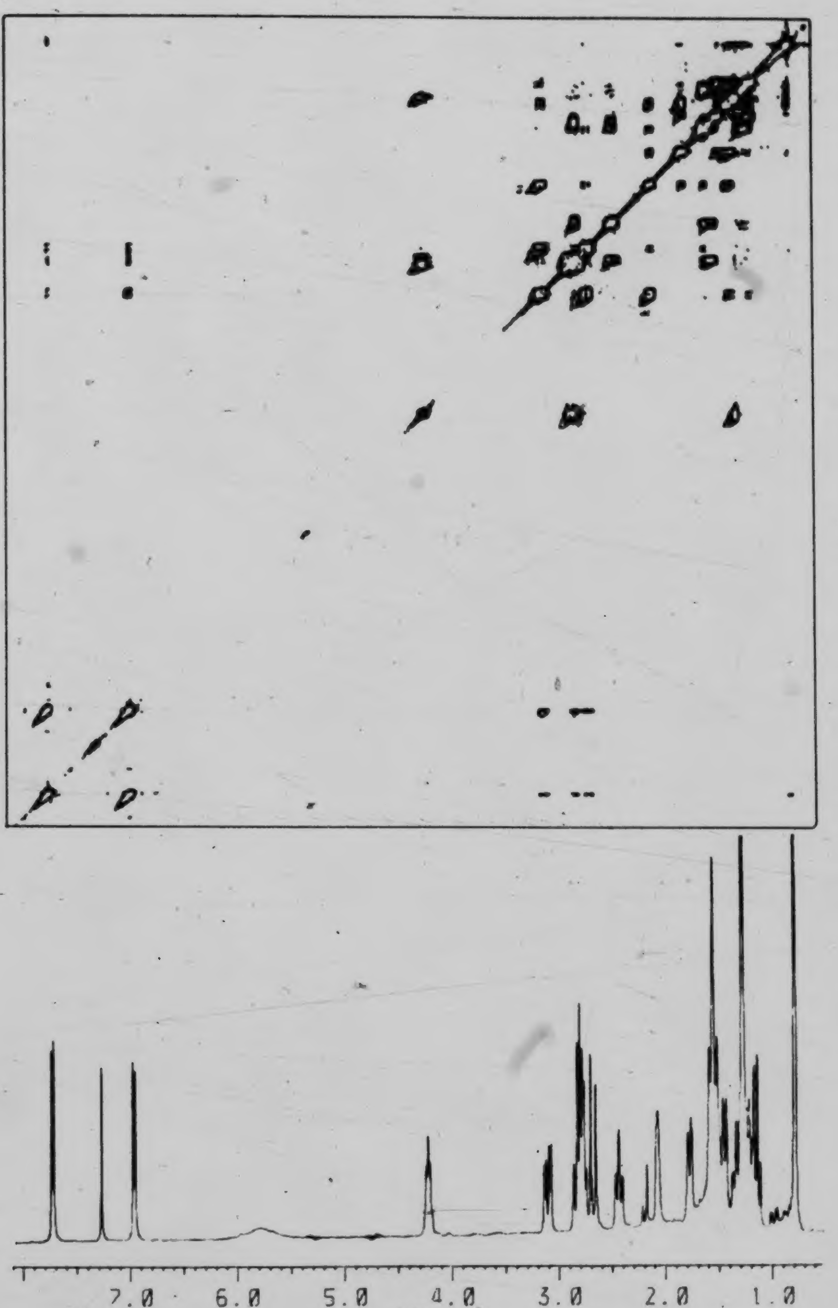
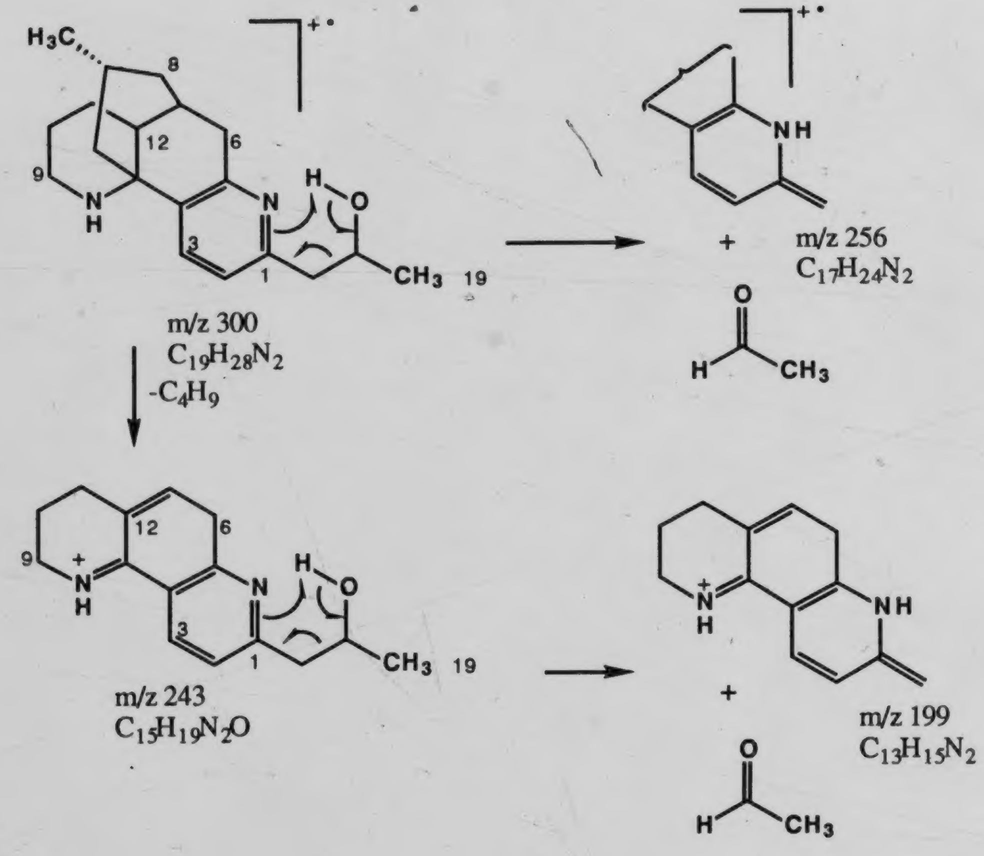


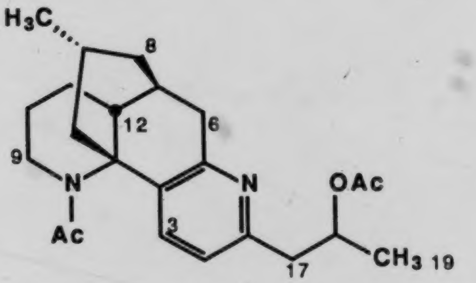
Figure 10. COSY 90 spectrum of hydroxypropyllycodine (36) ($CDCl_3$, 360 MHz).

In its hreims hyroxypropyllycodine (**36**) shows a base peak at m/z 243 ($C_{15}H_{19}N_2O$) which arises from loss of 57 (C_4H_9) units, as is commonly observed in the mass spectrum of some Lycopodium alkaloids[30]. The ion at m/z 256 ($C_{17}H_{24}N_2$) is due to loss of acetaldehyde from the parent ion, while the ion at m/z 199 ($C_{13}H_{15}N_2$) arises from loss of acetaldehyde from the base peak as shown in scheme 9.


H3C
8
12
6
9
N
H
N
H
O
3
1
CH3 19
m/z 300
C19H28N2
-C4H9
NH
+
m/z 256
C17H24N2
O
H
CH3
12
6
9
+
N
H
N
H
O
3
1
CH3 19
m/z 243
C15H19N2O
+
N
H
NH
+
m/z 199
C13H15N2
O
H
CH3

Scheme 9. Fragmentation in the hreims of hydroxypropyllycodine (**36**).

Upon acetylation, hydroxypropyllycodine (36) gave diacetate 37 ($C_{23}H_{32}N_2O_3$, 384.2305 from hreims). The ftir spectrum of the diacetate shows the absence of NH and OH, but reveals presence of an acetoxyl (1737 cm^{-1}) and N-acetyl (1656 cm^{-1}) groups.



37

In the ^1H nmr spectrum of diacetate 37 downfield shifts are observed for the carbinyl hydrogens at C-18 (δ5.27) and the methylene hydrogens at C-9 (δ3.60 and 2.63), C-17 (δ3.03 and 2.91), and C-14 (δ3.17 and 1.79). These hydrogens have shifted from the signals at δ4.21, 2.77 and 2.43, 2.89 and 2.71, and 1.43 and 1.13, respectively, in the ^1H nmr spectrum of 36. The chemical shift assignments are based on an analysis of the COSY 90 spectrum of diacetylhydroxypropyllycodine (37) (figure 11). The signal at δ3.17 (brdm, J = 12 Hz) is unambiguously assigned to the *equatorial* hydrogen at C-14. It is not possible to assign this signal from 1D ^1H nmr. However, bold lines in the expanded spectrum (figure 12) show that the methyl group at C-16 (δ0.81) is coupled to the methine hydrogen at C-15 (δ1.18), which is further coupled to the methylene hydrogens at C-14 (δ3.17 and 1.17). The *equatorial* hydrogen (δ3.17) is shifted downfield (Δ1.74) due to the deshielding effect of the N-acetyl carbonyl group.

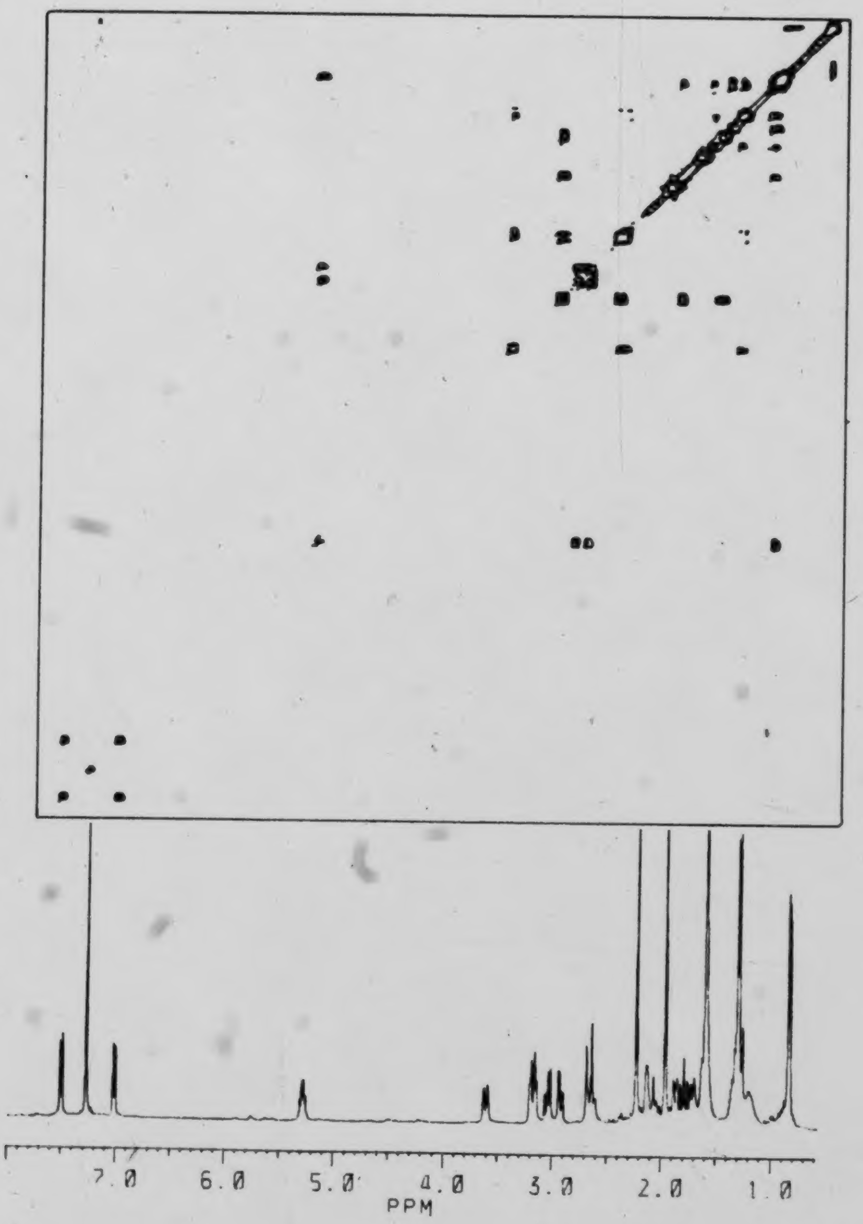


Figure 11. COSY 90 spectrum of diacetylhydroxypropyllycodine (37) ($CDCl_3$, 360 MHz).

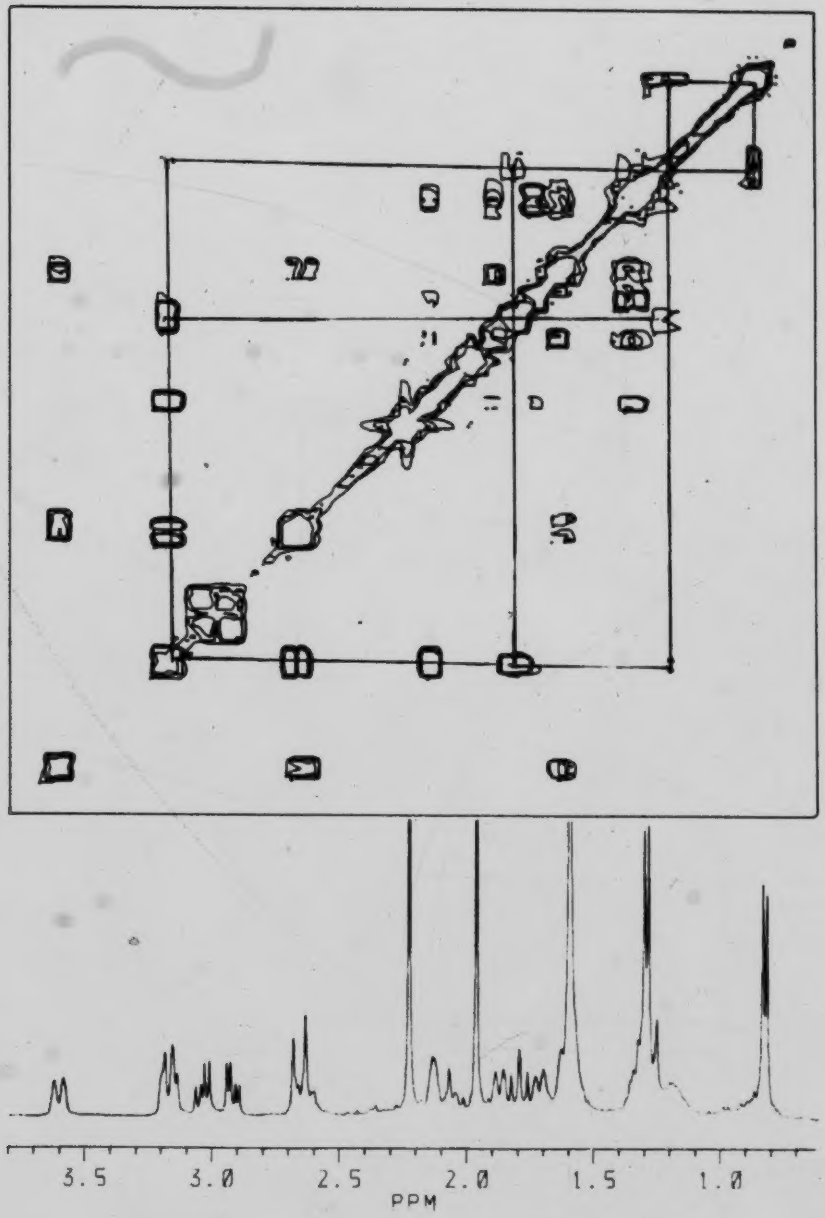
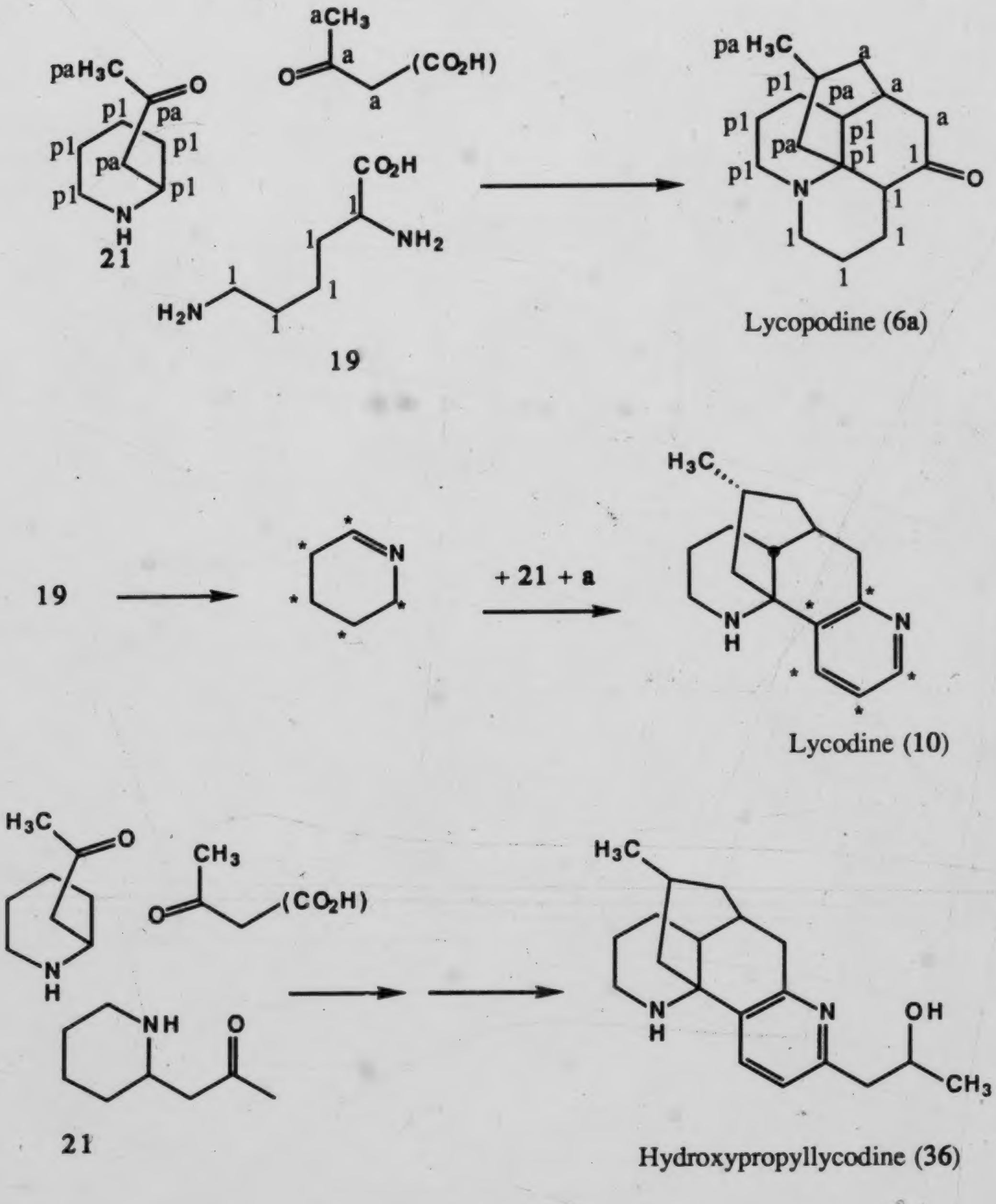


Figure 12. COSY 90 expansion spectrum of diacetylhydroxypropyllycodine (37).


paH₃C
O
pl
pa
pl
pl
pa
pl
pl
N
H
21
aCH₃
a
(CO₂H)
O
a
CO₂H
NH₂
H₂N
1
1
1
1
1
1
19
pa H₃C
pl
pa
a
a
a
pl
pa
pl
pl
pl
1
O
N
1
1
1
1
Lycopodine (6a)
19
N
+ 21 + a
H₃C
N
H
N
Lycodine (10)
H₃C
O
CH₃
(CO₂H)
O
N
H
NH
O
21
H₃C
N
H
N
OH
CH₃
Hydroxypropyllycodine (36)

Scheme 10. Summary of the biogenesis of some Lycopodium alkaloids.

Hydroxypropyllycodine is the first encountered Lycopodium alkaloid with a nineteen carbon skeleton and thus is biogenetically very interesting. As indicated in scheme 10, the skeleton can be constructed from two pelletierine units and one (decarboxylated) acetoacetate unit.

Des-N-methyl β-obscurine (12d).

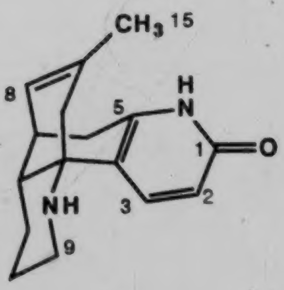
The more polar fraction of crude *L. obscurum* precipitates base **12d** as white granules upon trituration with cold acetone. Base **12d** crystallizes from acetone-methanol as colourless needles mp 290-292.5°C, and is optically active ($[\alpha]_D = -34^o$).

In its hreims base **12d** shows a molecular ion, m/z 258.1733 ($C_{16}H_{22}N_2O$). The base peak is at m/z 201 ($C_{12}H_{13}N_2O$) arising from loss of a C_4H_9 unit, the characteristic fragmentation observed in many Lycopodium alkaloids[30]. None of the nitrogen atoms in the molecule are lost, suggesting that both nitrogens are present as part of a ring system. The presence of ions at m/z 215 ($C_{13}H_{15}N_2O$) and m/z 173 ($C_{10}H_9N_2O$) arising from loss of a C_3H_7 unit from the parent ion and a C_2H_4 unit from the base peak, respectively is also typical of certain Lycopodium alkaloid series.

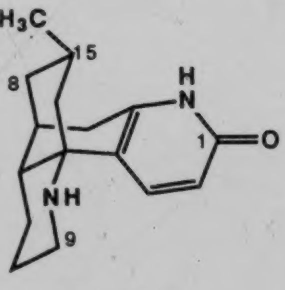
In the ftir spectrum of **12d**, sharp medium intensity absorptions at 3302 and 3021 cm^{-1} are consistent with the presence of an NH and an olefinic CH group, respectively. Sharp and strong absorptions at 1659, 1623, and 1554 cm^{-1} are consistent with the presence of an α-pyridone ring[31]. Absorptions in the uv spectrum (λ_{max} 230 (3.8) and 314 (3.7) nm) and ^{13}C nmr spectrum (δ 164.7(s), 145.5(s), 134.9(d), 117.9(s) and 117.5(d)) support the presence of an α-pyridone ring. The presence of two *ortho* related hydrogens (δ 7.57 and 6.44, J = 9 Hz) in the ^1H nmr spectrum reveals that the pyridone ring is disubstituted. Huperzine A (**1**) and B (**2**) which also possess an α-pyridone ring show similar uv and ir data[8].

The ^{1}H nmr spectrum accounts for twenty of the twenty two hydrogens in the formula. Since the ^{1}H nmr of base **12d** is apparently unchanged on D_2O exchange, the missing two hydrogens are too broad to be observed. This information is consistent with the presence of NH hydrogens. The large magnitude of *geminal* coupling (J = 19 Hz) between the methylene hydrogens (δ 2.91 and 2.40) is consistent with a methylene group at C-6, α to an sp^2 hybridized center at C-5 of the α-pyridone ring. The C-6 methylene hydrogen (δ 2.91) is coupled to a methine hydrogen (δ 2.01 br sextet, J = 3 Hz). Irradiation of H-6 (δ 2.91) simplifies the signal at δ 2.40 (d, J = 19 Hz) to a singlet, while the methine hydrogen (δ 2.01) collapses to a broad quintet. Careful inspection of the remaining signals in the ^{1}H nmr spectrum (see experimental) indicates similarity to the ^{1}H nmr spectrum of lycodine (**10**) and hydroxypropyllycodine (**36**).

The ^{13}C APT spectrum of base **12d** shows signals for the sixteen carbons present in its formula (11 quaternary / CH_2 signals and 5 CH_3 / CH signals). The carbon chemical shifts are similar to those reported for huperzine B (2)[8] (table 14). Base 12d differs from huperzine B at C-14 and C-15, which are sp^2 hybridized in huperzine B and sp^3 hybridized in **12d**. These data suggest that base **12d** is des-N-methyl-β-obscurine. Des-N-methyl-β-obscurine has previously been prepared in these laboratories[16b]. The natural product is identical with the sample prepared from β-obscurine (ir, uv, and ^{1}H nmr). To our knowledge this is the first report of its occurrence in nature.



2



12d

Table 14 Comparison of the carbon 13 nmr spectra of des-N-methyl-β-obscurine (12d) and huperzine B (2).

		Chemical shift in δ.	
C	mult.	2 (25.18 MHz)	12d (100.6 MHz)
1	s	165.4	164.7
2	d	117.9	117.5
3	d	140.4	139.9
4	s	117.9	117.9
5	s	143.3	144.5
6	t	29.45	30.1
7	d	34.59	33.4
8	d	126.1	43.2(t)[a]
9	t	48.03	49.6
10	t	25.3	25.8
11	t	28.1	27.9
12	d	40.7	44.7
13	s	53.2	54.5
14	t	41.7	41.3[a]
15	s	132.2	29.5(d)
16	q	22.7	21.9

a, signals may be reversed.

Lyconnotinol (38).

Preparative tlc of the more polar (lower Rf) chromatography fractions afforded a minor base, compound **38**, isolated as the major component of a mixture containing some other minor alkaloids. Base **38** decomposes on standing exposed to air to a more polar reddish brown material. This may be separated from base **38** by filtration through alumina (methanol-dichloromethane). A pure sample of the alkaloid was obtained as its monoacetyl derivative **39**.

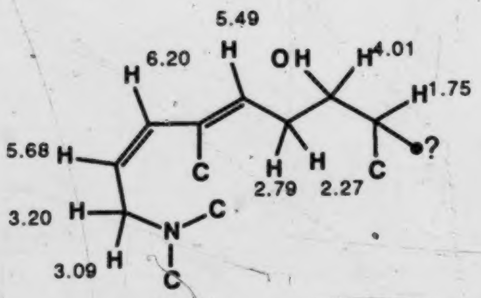
The molecular weight 263 ($C_{16}H_{25}NO_2$) for base **38** was deduced by a combination of gc / ms, hreims, and cims. The peak at m/z 264 (100%) in cims corresponds to a protonated form of base **38**. The parent ion was not evident in the gc/ms or in the hreims. The monoacetyl derivative **39**, however, indicates a parent ion at m/z 305 ($C_{18}H_{27}NO_3$). The gc / ms of base **38** displays peaks arising from loss of a molecule of water (m/z 245) and loss of hydroxyl group plus a molecule of water(m/z 288). This indicates that the oxygens in the molecular formula of **38** exist as two hydroxyl functionalities. The primary nature of one of the hydroxyl groups is evident from the ^1H nmr spectrum of **38**. An AB pattern observed at δ 3.42 and 3.28 is consistent with the presence of a methylene group *geminal* to a primary hydroxyl group. The AB pattern in the ^1H nmr spectrum of monoacetate **39** is shifted downfield by *ca* Δ0.41 as expected for a primary alcohol[32].

In the ^1H nmr spectrum of monoacetate **39** absorptions at δ6.20, 5.68, and 5.49 are attributed to olefinic hydrogens, while absorptions at δ4.10 and 1.38 are due to a carbinyl and hydroxyl hydrogen, respectively. On addition of D_2O, the signal at δ4.10 (m) simplifies to a double doublet (J = 7.5,3.4 Hz) and the signal at δ1.38 (brd, J = 3.3 Hz) disappears.

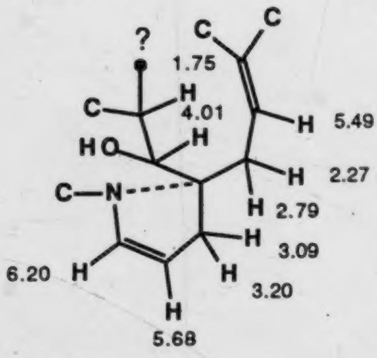
The ^{13}C APT spectrum of the monoacetate **39** shows eighteen carbons (10 quaternary / CH_2 signals and 8 CH_3 / CH signals). There are five sp^2 hybridized carbons including the acetoxyl carbonyl (δ 171.2 (s), 137.6 (s), 128.3 (d), 124.6 (d), and 122.9 (d)). The signals at δ 69.2 (d) and 69.0 (t) in ^{13}C nmr spectrum are due to carbons bearing a secondary and primary hydroxyl group, respectively. Three olefinic hydrogens plus four sp^2 hybridized carbons are consistent with two carbon-carbon double bonds in the molecule. Five sites of unsaturation are suggested by the molecular formula. Since there are two double bonds base **38** must have a tricylic structure. Absorption in the uv spectrum at λ_{max} 235 (3.7) nm indicates either that the double bonds exist as a *transoid* conjugated diene[33] or that one of the double bonds is α to a nitrogen atom[22].

^1H nmr studies with monoacetate **39** (figure 13) revealed the ^1H-^1H connectivities. The three spin systems I, II, and III (scheme 11) were derived by analysis of the COSY 90 spectrum.

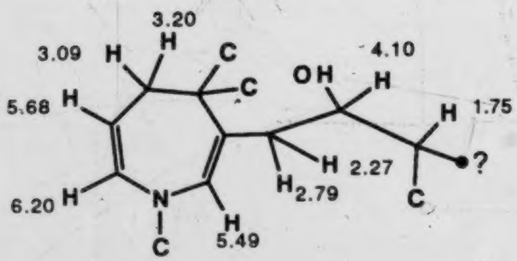
Consideration of the uv and nmr data led to the three possible partial structures A-1, A-2, and A-3. The *cis* related olefinic hydrogens (δ 6.20 and 5.68) each show coupling to an AB quartet (δ 3.20 and 3.09). The hydrogen resonating at δ 3.20 shows long range coupling to a methylene hydrogen (δ 2.27), whose *geminal* partner resonates at δ 2.79. Each of the methylene hydrogens (δ 2.27 and 2.79) show coupling to the olefinic hydrogen (δ 5.49). The hydrogen resonating at δ 2.79 is further coupled to the carbinyl hydrogen (δ 4.10), which is in turn coupled to the hydroxyl hydrogen (δ 1.38) and to a signal centered at ca δ 1.75 (see slice from the COSY 90 spectrum centered at δ 4.10, figure 14). Partial structure A-3 was discarded on the basis of the coupling constant between the carbinyl hydrogen (δ 4.10) and the hydrogen resonating at δ 2.79, J = 7.5 Hz. Partial structure A-3 requires a four bond coupling of 7.5 Hz, which is unlikely[34].


5.49
6.20
4.01
1.75
5.68
3.20
3.09
2.79
2.27
?
OH
N

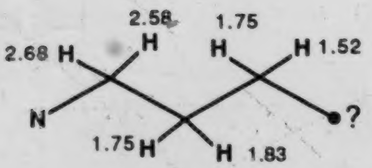
(A-1)


?
1.75
4.01
5.49
2.27
2.79
3.09
3.20
6.20
5.68
HO
N

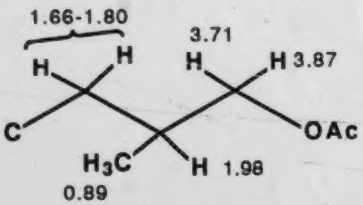
(A-3)


3.20
3.09
4.10
5.68
1.75
2.27
2.79
6.20
5.49
?
OH
N

(A-2)

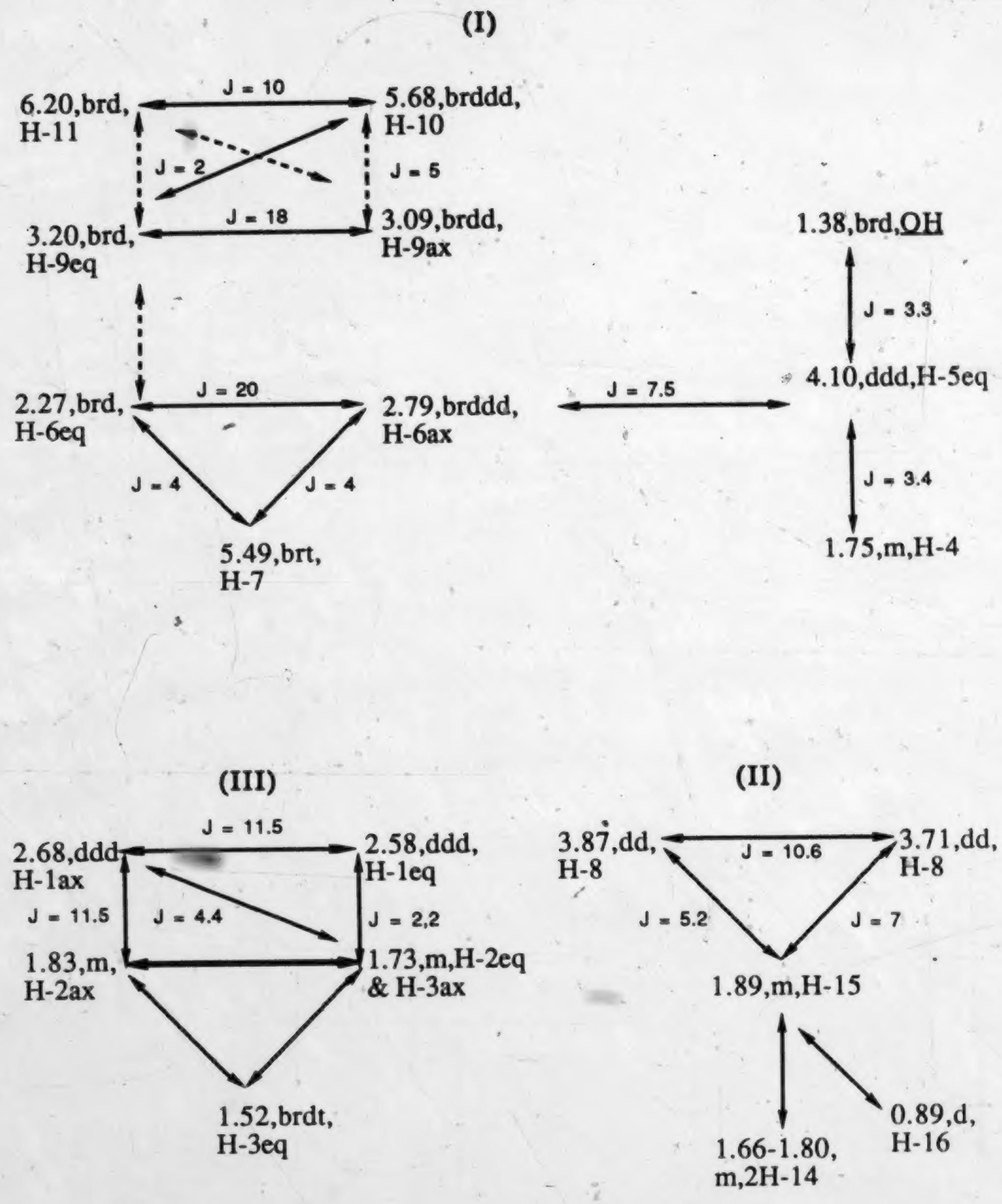

2.58
1.75
2.68
1.52
1.75
1.83
N
?

(C)


1.66-1.80
3.71
3.87
OAc
H₃C
0.89
1.98

(B)

Partial structure B was evident from correlations of spin system II. The hydrogens of an AB quartet (δ 3.87 and 3.71) are coupled to a methine hydrogen (δ 1.98), which is further coupled to a methyl (δ 0.89) and a methylene group resonating in the multiplet (δ 1.66-1.80) (see slice from the COSY 90 spectrum centered at δ 1.98, figure 14). The hreims of **38** and **39** provides support for partial structure B. The base peak at m/z 190 arises from loss of partial structure B as C_4H_9O in **38** and as $C_6H_{11}O_2$ in **39**.


(I)
6.20,brd,
H-11
J = 10
5.68,brddd,
H-10
J = 2
J = 5
3.20,brd,
H-9eq
J = 18
3.09,brdd,
H-9ax
1.38,brd,OH
J = 3.3
4.10,ddd,H-5eq
J = 7.5
2.27,brd,
H-6eq
J = 20
2.79,brddd,
H-6ax
J = 4
J = 4
J = 3.4
5.49,brt,
H-7
1.75,m,H-4
(III)
J = 11.5
2.68,ddd,
H-1ax
2.58,ddd,
H-1eq
J = 11.5
J = 4.4
J = 2,2
1.83,m,
H-2ax
1.73,m,H-2eq
& H-3ax
1.52,brdt,
H-3eq
(II)
3.87,dd,
H-8
J = 10.6
3.71,dd,
H-8
J = 5.2
J = 7
1.89,m,H-15
0.89,d,
H-16
1.66-1.80,
m,2H-14

Scheme 11. ^1H-^1H chemical shift correlations obtained from the COSY 90 spectrum of monoacetyllyconnotinol (39).

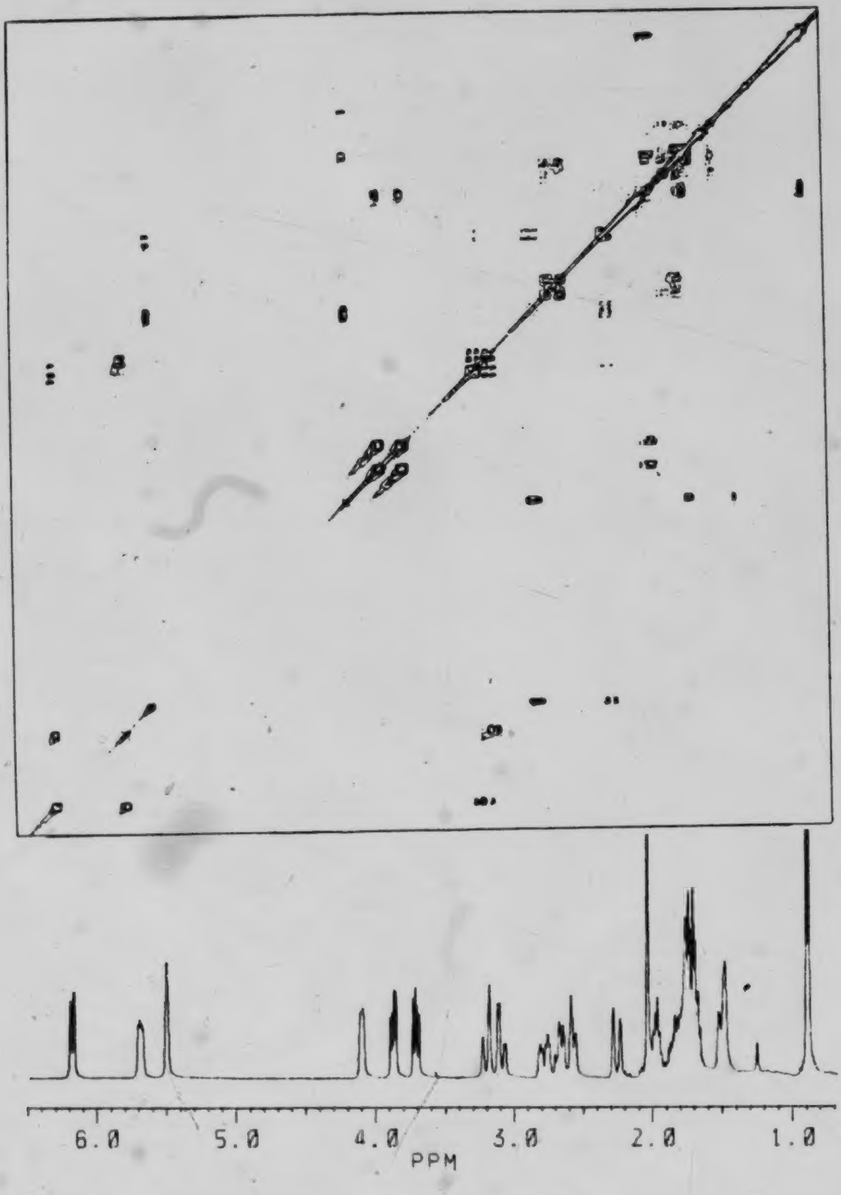


Figure 13 COSY 90 spectrum of monoacetyllyconnotinol (39) ($CDCl_3$, 360 MHz).

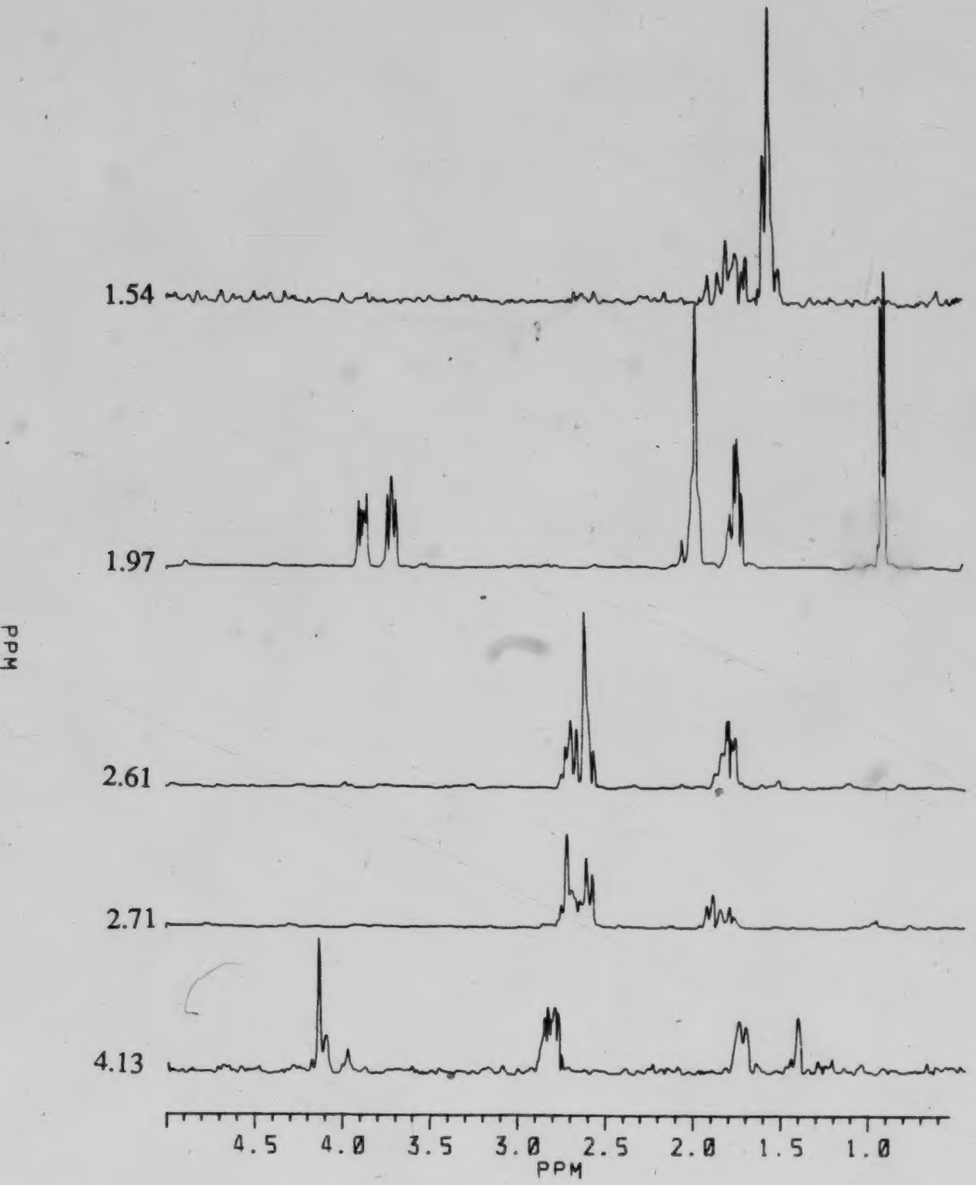
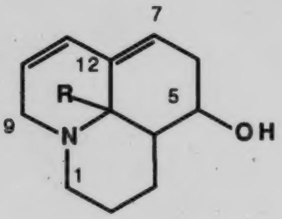


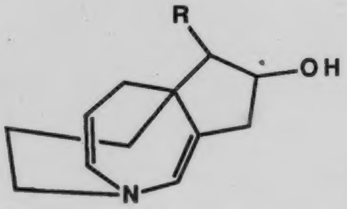
Figure 14 Slices from the COSY 90 spectrum of monoacetyllyconnotinol (39).

Partial structures A-1 or A-2 and B account for fourteen of the eighteen carbons in the ^{13}C nmr spectrum (2 quaternary, 2 CH_3, 4 CH_2, and 6 CH signals). With the exception of one signal at δ55.9 (quaternary carbon), the remaining three CH_2 signals are accounted for in correlations of spin system III, which gives rise to partial structure C.

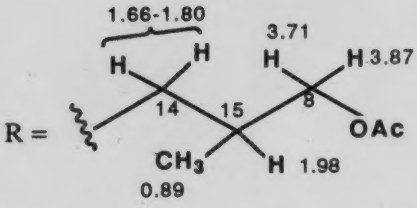
Structure **39** was formulated from partial structures A-1, B, and C and structure **40** was formulated from partial structures A-2, B, and C. Structure **40** was disfavored since in **40** the lone pair on the nitrogen atom is not orthogonal to any of the carbon-carbon double bonds and thus could not account for the uv spectrum. Also, structure **40** does not account for an easy loss of the hydroxylisobutyl group (partial structure B) to afford a base peak at m/z 190 in the hreims.



39



40



Support for structure **39** was provided by NOESY and nOe experiments. The NOESY spectrum (figure 15) verified the *geminal* partners for methylene groups. Stronger dipolar couplings (indicated by solid lines) are observed between *geminal*

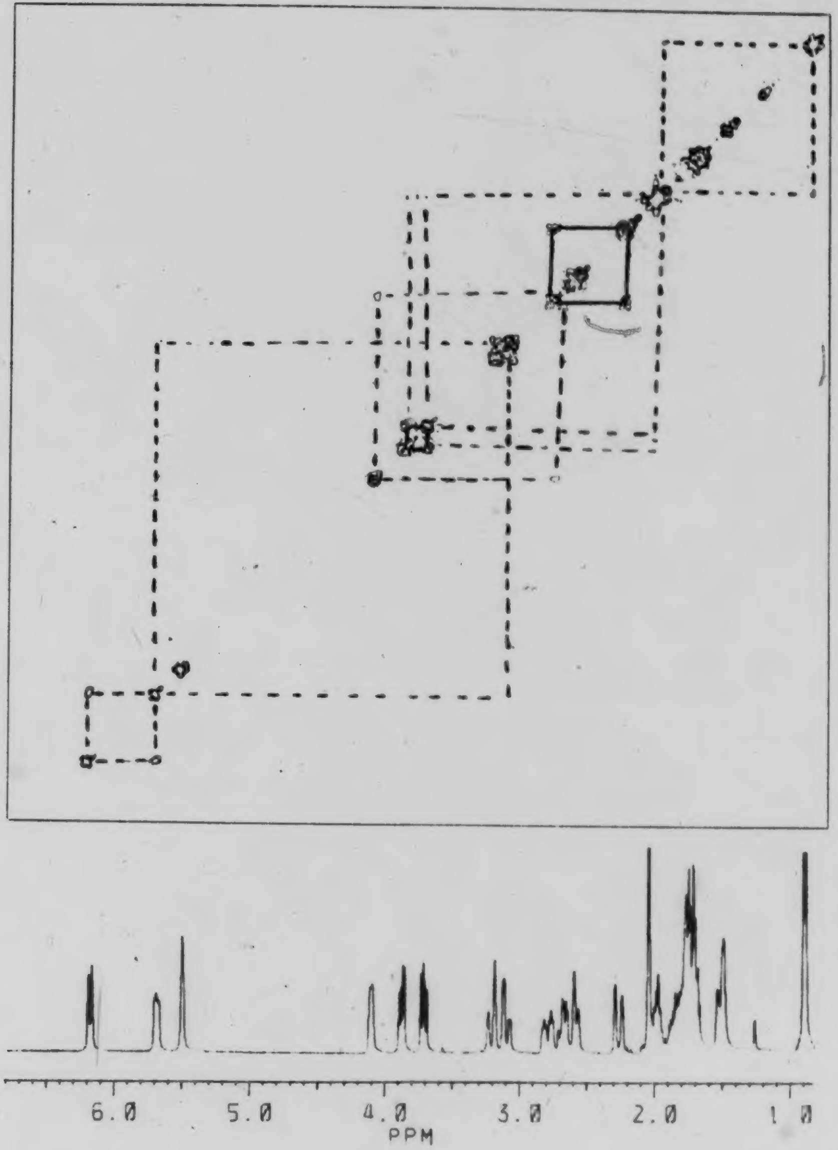


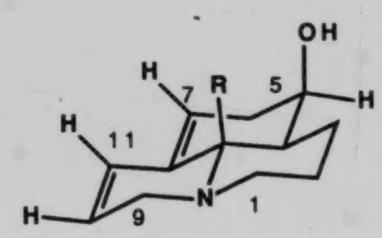
Figure 15 NOESY 45 spectrum of monoacetyllyconnotinol (39) (CDCl$_3$, 400 MHz).

partners than between any other pair (indicated by dashed lines). In the ^{1}H nOe experiments signal enhancements upon irradiation of various hydrogens are as shown in table 15.

Table 15 ^{1}H nmr nOe experiments with monoacetyllyconnotinol (39) ($CDCl_3$, 360 MHz).

Chemical shift in δ.	
Signal saturated	**Observed change(%nOe)**
6.2(H-11)	5.68(H-10)(6.5)
	5.49(H-7)(6.5)
5.68(H-10)	6.20(H-11)(7.6)
	3.20/3.03(2H-9)(3.4)
5.49(H-7)	6.20(H-11)(7.6)
	2.79(H-6ax)(2.5)
	2.27(H-6eq)(2.5)
4.10(H-5eq)	2.79(H-6ax)(3.7)
	1.80-1.66(H-4ax)(12.9)
	1.38(OH)(8.6)
3.87(H-8a)	3.71(H-8b)(20.6)
	1.98(H-15)(7.4)
3.71(H-8b)	3.87(H-8a)(17.3)
	1.98(H-15)(4.7)
2.79(H-6ax)	5.49(H-7)(3.4)
	4.01(H-5eq)(3.4)
	2.27(H-6eq)(17.4)
2.27(H-6eq)	2.79(H-6ax)(12.3)
1.38(-OH)	4.01(H-5eq)(7.6)
0.89(H-16)	1.98(H-15)(13.0)
	1.80(H-14)(8.0)

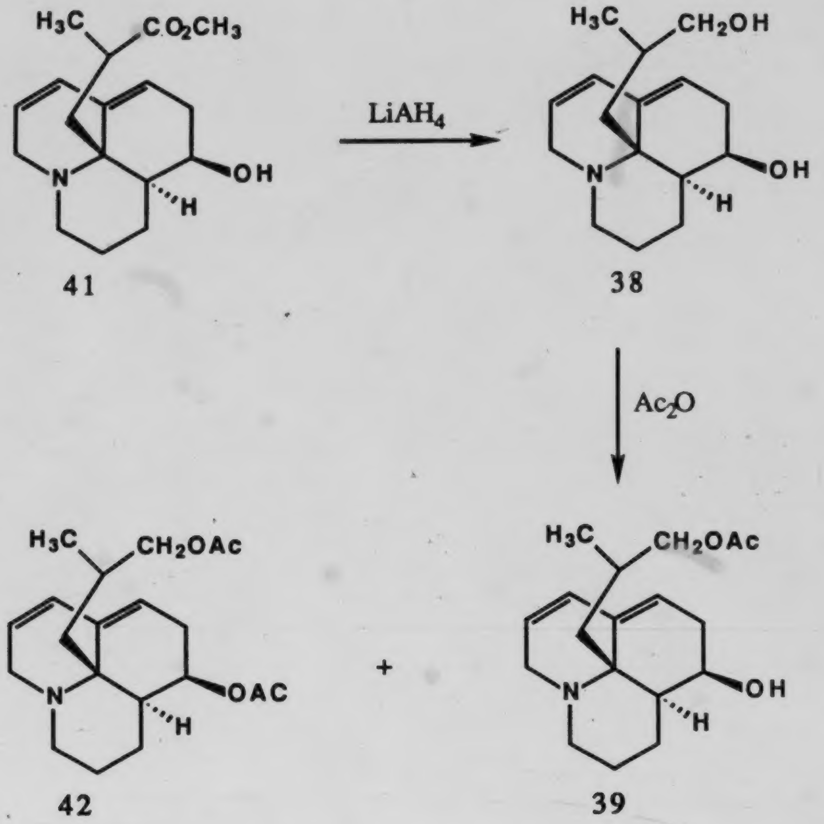
The hydroxyl group at C-5 is *axial* and hindered as required by the acetylation experiment where treatment of base **38** with acetic anhydride at room temperature effected acetylation almost exclusively at the primary hydroxyl group. Acetylation at C-5 occurred only to a very small extent.

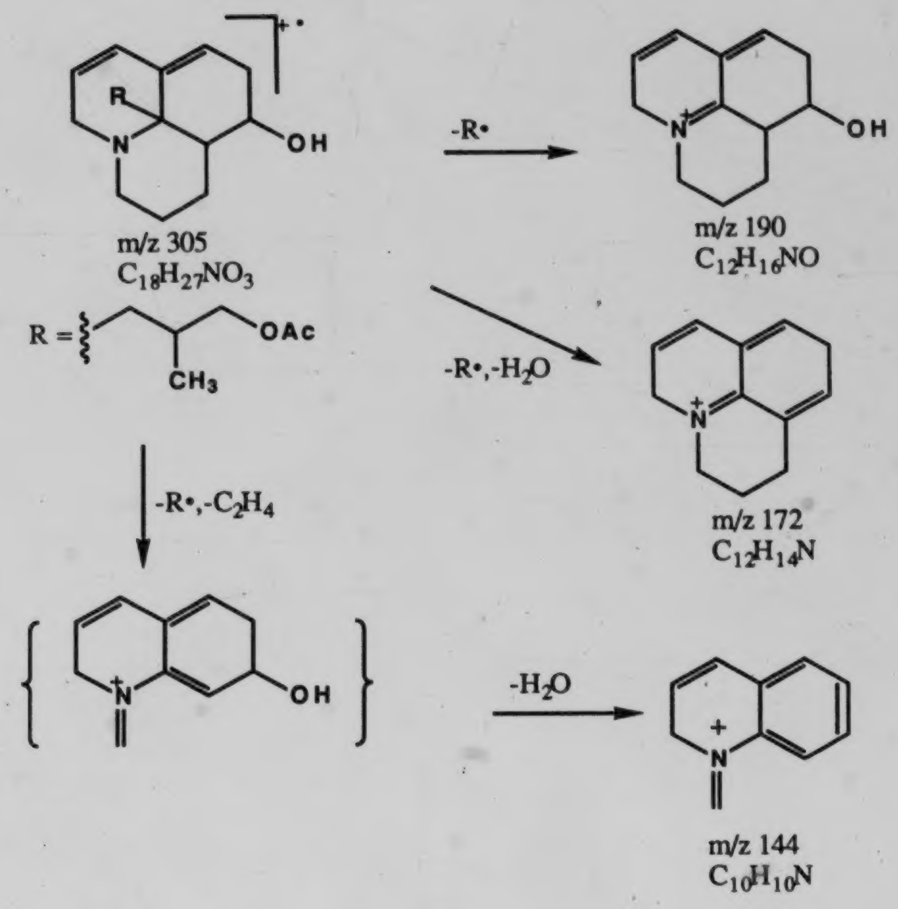


39

A fragmentation pattern in the hreims consistent with structure **39** is as shown in scheme 12. Compound **39** is related to a known alkaloid, lyconnotine (**41**)[35]. To verify structure 39 lyconnotine (41) was reduced with lithium aluminium hydride to give a diol. The diol **38** obtained was identical with the naturally occurring base **38** (^{1}H and ^{13}C nmr spectra). Acetylation of synthetic diol **38** afforded the mono and diacetyl derivatives 39 and 42, respectively. The derived monoacetate 39 was also identical with that from the natural product (^{1}H and ^{13}C nmr spectra). We therefore suggest the name lyconnotinol for base 38.

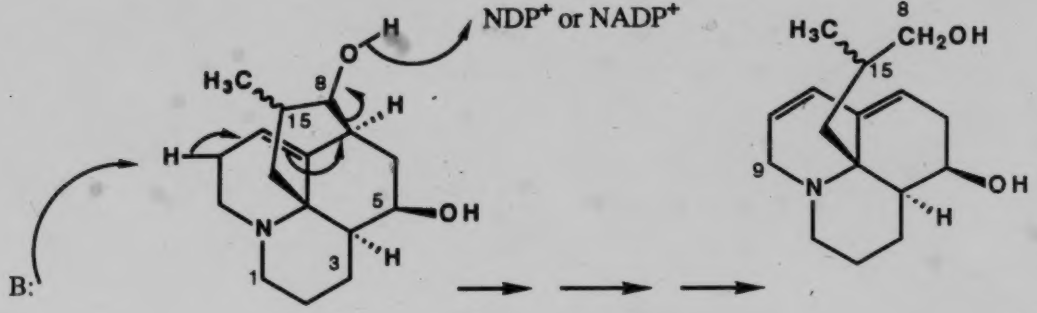
The carbon-chemical shift assignments for lyconnotine (41), lyconnotinol (38), monoacetyllyconnotinol (39), and diacetyllyconnotinol (42) based on ^{13}C APT experiments are shown in table 16. It is not possible to unambiguously distinguish C-7 from C-10 and C-2 from C-3. A ^{1}H-^{13}C correlation experiment was carried out on monoacetyllyconnotinol (**39**) (figure 16). There is a cross peak between H-10 (δ 5.68) and C-10 (δ 124.6) and between H-3 (δ 1.52) and C-3 (δ 22.7).


H₃C
CO₂CH₃
N
OH
H
41
LiAH₄
H₃C
CH₂OH
N
OH
H
38
Ac₂O
H₃C
CH₂OAc
N
OAC
H
42
+
H₃C
CH₂OAc
N
OH
H
39


m/z 305
$C_{18}H_{27}NO_3$
R =
OAc
CH3
OH
N
-R•
m/z 190
$C_{12}H_{16}NO$
-R•,-H2O
m/z 172
$C_{12}H_{14}N$
-R•,-C2H4
-H2O
m/z 144
$C_{10}H_{10}N$

Scheme 12. Fragmentation in the hreims of monoacetyllyconnotinol (**39**).

A possible biogenesis for lyconnotinol (**38**) or lyconnotine (**41**) from lycofoline (**8**) or acrifolinol (**43**) (structures *vide infra*) is shown in scheme 13 (lycofoline (**8**) and acrifolinol (**43**) are C-15 epimers). The absolute configuration at C-15 of lyconnotinol or lyconnotine still remains unsolved. Knowledge of the absolute configuration at C-15 of lyconnotinol or lyconnotine would provide a clue as to whether **8** or **43** is a precursor in the biogenesis of **38** and **41**. This is the second report of this particular skeleton among the Lycopodium alkaloids. Lyconnotine is the other example.



43 or **8**

38

Scheme 13. A possible biogenesis of lyconnotinol (**38**) from acrifolinol (**43**) or lycofoline (**8**).

Table 16 ^{13}C nmr spectra of lyconnotine (41), lyconnotinol (38), monoacetyllyconnotinol (39), and diacetyllyconnotinol (42). ($CDCL_3$, 100.6 MHz).

		Chemical shift in δ.			
C	mult.	41	38	39	42
1	t	48.2	47.7	48.0	47.7
2	t	25.8	25.6	26.0	25.7
3	t	23.4	22.8	22.7	22.6
4	d	47.5	48.0	48.3	46.7
5	d	69.4	69.2	69.3	71.2
6	t	35.4	34.6	34.8	32.0
7	d	123.0	122.7	122.9	122.2
8	t	178.3(s)	69.0	70.5	70.3
9	t	49.3	49.7	49.8	49.8
10	d	124.7	124.3	124.6	124.7
11	d	127.1	128.4	128.3	128.1
12	s	136.8	138.0	137.6	137.6
13	s	56.1	56.2	55.9	55.6
14	t	27.6	27.1	26.8	26.6
15	d	37.2	32.6	29.5	29.4
16	q	21.0	19.7	20.0	20.1
17	q	51.2			
$\underline{C}OCH_3$	s			171.2	170.7/171.2
$COC\underline{H}_3$	q			21.0	21.0/21.4

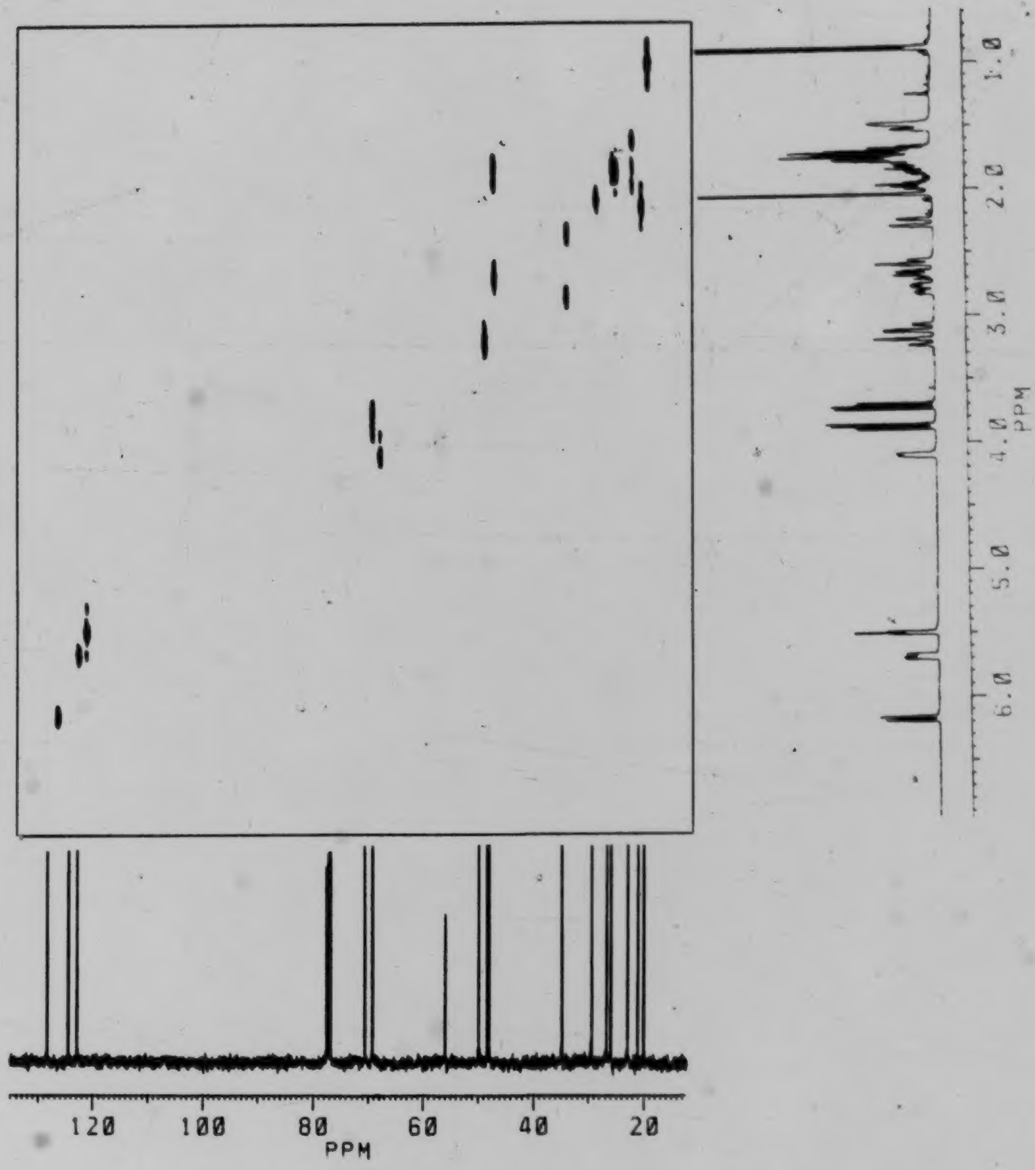


Figure 16 ^{1}H-^{13}C COSY spectrum of monoacetyllyconnotinol (39) ($CDCl_3$, 400 MHz).

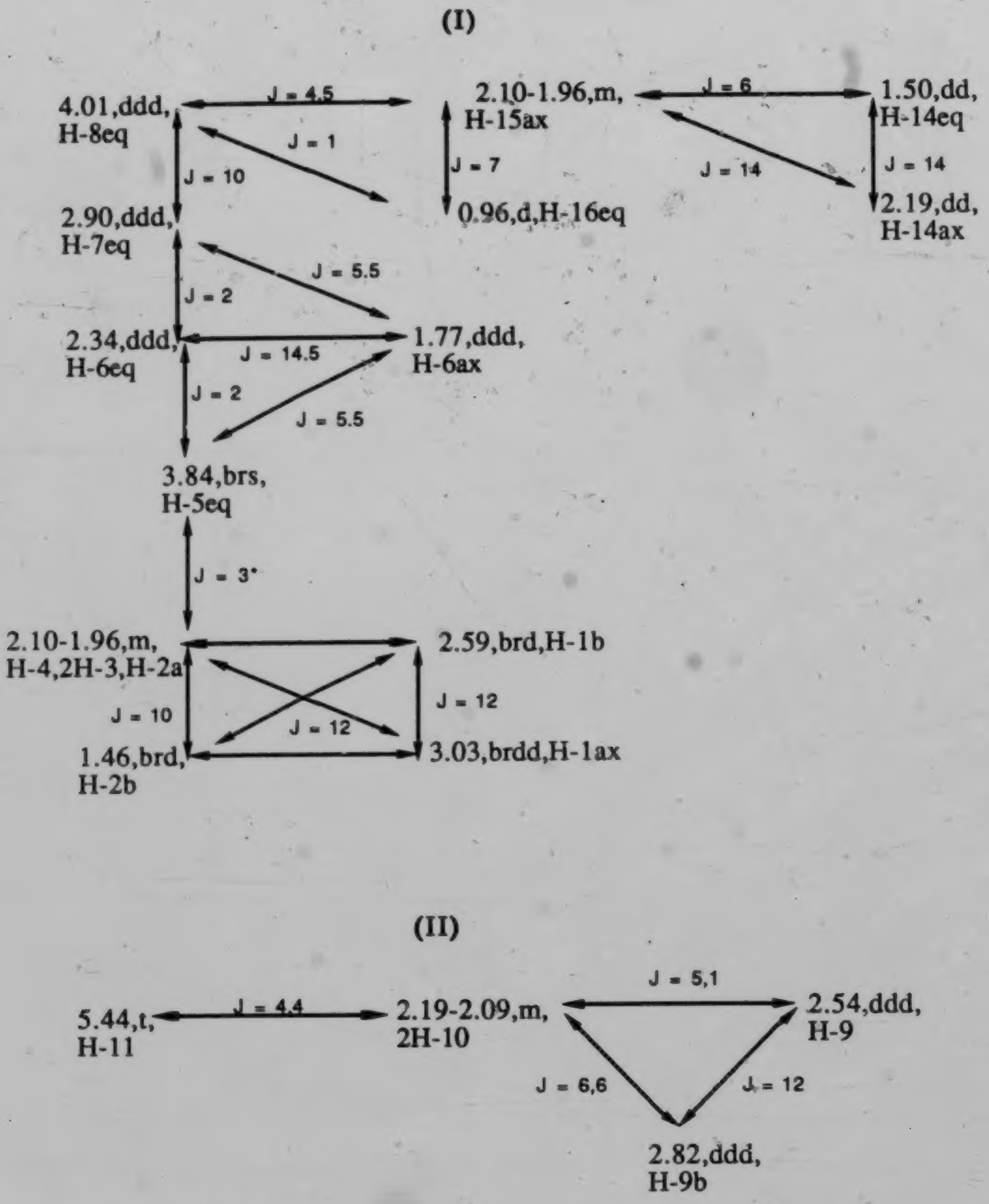
Acrifolinol (43) and Lycofoline (8)

Fractions containing lycoannotinol (38) also afforded two minor, closely related, isomeric alkaloids **43** and **8**. The hreims spectra of both **43** and **8** indicate a molecular formula, $C_{16}H_{25}NO_2$ (263) and cims spectra confirm the molecular weight of each. The hreims of both compounds display identical fragmentation patterns consistent with a lycopodane skeleton.

The ^{1}H nmr spectrum of base **43** shows signals characteristic of an olefinic hydrogen (δ5.44), two carbinyl hydrogens (δ4.01 and 3.84), and a secondary methyl group (δ0.96). The ^{13}C APT experiment with base **43** indicates the presence of two sp^2 hybridized carbons (δ146.3, s and 116.4, d) and two carbons bearing secondary hydroxyl groups (δ73.5, d and 69.6, d). The absorption bands at 3200 and 1052 cm^{-1} in the ftir spectrum of **43** confirm the presence of hydroxyl groups. Five sites of unsaturation in the molecular formula are consistent with a tetracyclic skeleton.

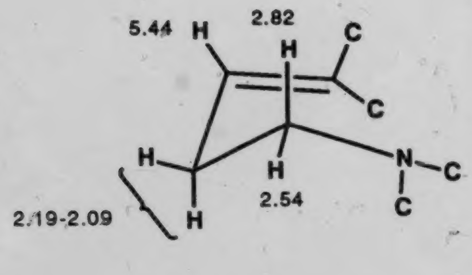
A 2D ^{1}H-^{1}H COSY experiment with base **43** indicates the presence of spin systems I and II (scheme 14). These spin systems as well as ^{13}C nmr spectral data (table 18) allow derivation of partial structures A and B. Combining these partial structures gives rise to structure **43**.

The relative stereochemistry and conformation of compound **43** is revealed from decoupling experiments and from the coupling pattern of the hydrogens as shown in scheme 14. Beginning with the methylene hydrogens at C-14 the correlation is extended to C-1 through the carbinyl hydrogen at C-8 and C-5. The signal at δ2.19 (dd, J = 14,14 Hz) is due to H-14 with an *axial* orientation. Since $J_{14,15}$ = 14 Hz, H-15 must necessarily be *axially* oriented (i.e., the dihedral angle approaches 180°).

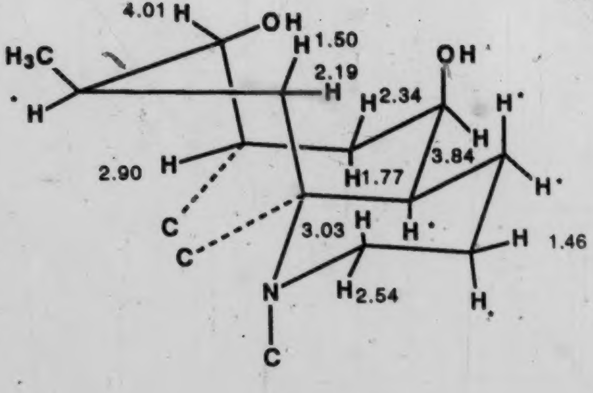

(I)
4.01,ddd,
H-8eq
J = 4.5
2.10-1.96,m,
H-15ax
J = 6
1.50,dd,
H-14eq
J = 1
J = 10
J = 7
J = 14
J = 14
2.90,ddd,
H-7eq
0.96,d,H-16eq
2.19,dd,
H-14ax
J = 5.5
J = 2
2.34,ddd,
H-6eq
J = 14.5
1.77,ddd,
H-6ax
J = 2
J = 5.5
3.84,brs,
H-5eq
J = 3*
2.10-1.96,m,
H-4,2H-3,H-2a
2.59,brd,H-1b
J = 10
J = 12
J = 12
1.46,brd,
H-2b
3.03,brdd,H-1ax
(II)
J = 5,1
5.44,t,
H-11
J = 4.4
2.19-2.09,m,
2H-10
2.54,ddd,
H-9
J = 6,6
J = 12
2.82,ddd,
H-9b

Scheme 14. ^1H-^1H chemical shift correlations obtained from the COSY 90 spectrum of acrifolinol (**43**).

* coupling constant J was deduced by decoupling experiment

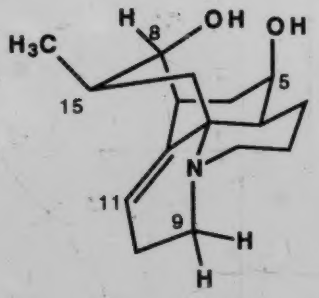

5.44 H
2.82
H
C
C
H
N—C
C
H
2.54
2.19-2.09
H

(A)


4.01 H
OH
H 1.50
H₃C
2.19
H
OH
H
H2.34
H*
2.90
H
H
3.84
H1.77
H*
C
C
3.03 H
H*
H 1.46
N
H2.54
H
C

(B)

Hydrogens marked with an asterisk appear in the multiplet at δ 2.10-1.96


H
8
OH
OH
H₃C
5
15
N
11
9
H
H

43

The carbinyl hydrogen at C-8 (δ4.10, ddd, J = 10,4.5,1 Hz) collapses to a broad doublet (J = 4.5 Hz), when H-7 (δ2.90 ddd, J = 10,5.5,2 Hz) is irradiated indicating that $J_{7,8}$ = 10 Hz and $J_{8,15}$ = 4.5 Hz. In accordance with the Karplus rule[24] H-8 must have an *equatorial* orientation, and the dihedral angle between H-7 and H-8 must approach 0°. This is possible if ring D is in a boat conformation as shown in structure 43. The coupling pattern of H-6eq (δ2.34 ddd, J = 15,2,2 Hz) and H-6ax (1.77, ddd, J = 15,5.5,5.5 Hz) indicate that H-5 and H-7 are *equatorial.* That H-5 is *equatorial* was confirmed by decoupling experiments. Upon irradiation of H-6eq (δ2.34, ddd, J = 15,2,2 Hz), H-7 (δ2.90, ddd, J = 10,5.5,2 Hz) collapses to a double doublet (J = 10,5.5

Hz), H-6ax (δ 1.77, ddd, J = 15,5.5,5.5 Hz) collapses to a double doublet (J = 5.5,5.5 Hz), and H-5 (δ 3.84, brs, $W_{1/2}$ = 10 Hz) simplifies to double doublet (*ca* J = 5,3 Hz). The magnitude of the coupling ($J_{5,4}$ = 3 Hz) precludes an *axial* orientation for H-5 since H-4 is α and *axial* (to the best of our knowledge there is no case where H-4 in a lycopodane type alkaloid is reported otherwise). The magnitude of the coupling of the methylene hydrogens at C-9 (δ 2.82, ddd, J = 12,6,6 Hz and 2.54, ddd, J = 12,5,1 Hz) and the lack of pronounced *Bohlmann* bands in the ftir spectrum of **43** indicate that ring A largely adopts a half boat conformation as shown in structure **43**.

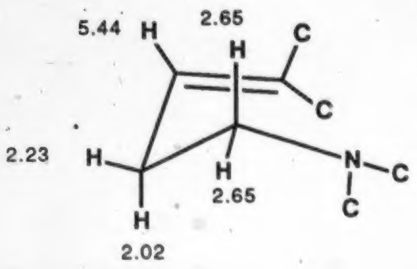
Support for structure **43** was provided by irradiation experiments in the nOe (see table 17). Irradiation of H-11 produced enhancement at H-7 and H-10β; H-7 to H-11, H-8, 2H-6, and H-5 and so forth as summarized in table 17. Compound **43** is identical with the known compound acrifolinol, the product of reduction of acrifoline[36]. To the best of our knowledge this is the first report of the occurrence of acrifolinol in nature.

Base **8**, is similar to acrifolinol in ftir, hreims, ^{1}H and ^{13}C nmr spectra. A 2D COSY 90 experiment with base **8** reveals two spin systems I and II as shown in scheme 15. Except for a very slight difference in chemical shift and coupling pattern for the hydrogens, the ^{1}H-^{1}H correlations are similar to those of base **43**. Partial structures A and B are also apparent from spin systems I and II, respectively. Structure **8** was proposed from partial structures A and B.

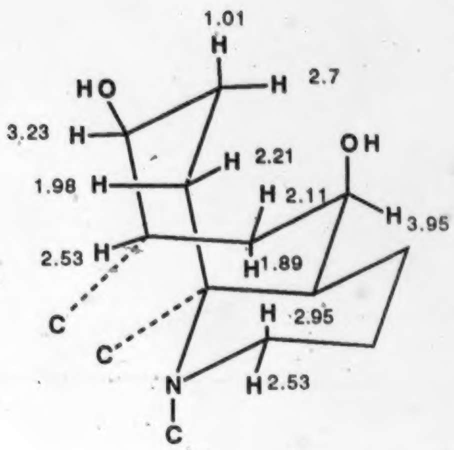
In the ^{1}H nmr of base **8** H-14 is *axially* oriented (δ 1.06, dd, J = 13,13 Hz) and this indicates that H-15 is also *axial.* Upon irradiation of the signal due to H-7 and H-1eq (δ 2.53, m), H-6eq (δ 2.11, brd, J = 15.5 Hz) sharpens, H-6ax (δ 1.89, ddd, J = 15.5,6,6 Hz) collapses to a double doublet (J = 15.5,6 Hz), and H-8 (δ 3.23, dd, J = 10,5.5 Hz) collapses to a doublet (J = 10 Hz) indicating that $J_{8,15}$ = 10 Hz. In accordance with the Karplus rule, H-8 is *axial* and thus ring D is in a chair conformation as shown in structure **8**.

Table 17 nOe experiments with acrifolinol (43) ($CDCl_3$, 360 MHz).

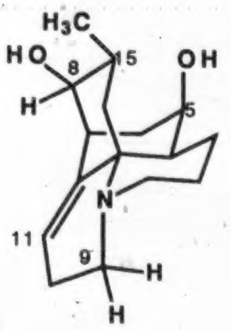
Chemical shift in δ.	
Signal saturated	**Signal enhanced(%nOe)**
5.44(H-11)	2.90(H-7eq)(7.4)
	2.10(H-10eq)(5.9)
4.01(H-8eq)	2.90(H-7eq)(6.8)
	1.9(H-15ax)(1.1)
3.84(H-5eq)	2.34(H-6eq)(3.7)
	1.77(H-6ax)(3.7)
3.03(H-1ax)	4.01(H-8eq)(-6.8)
	3.84(H-5eq)(-6.8)
	2.59(H-1eq)(17.1)
	1.96-2.0(H-2a/H-3)(-19.9)
	1.46,(H-2b)(2.7)
2.90(H-7eq)	5.44(H-11)(9.3)
	4.01(H-8eq)(-0.7)
	3.84(H-5eq)(-2.9)
	2.34(H-6eq)(2.4)
	1.72(H-6ax)(2.4)
0.96(H-16eq)	1.96(H-15ax)(9.9)


5.44
H
2.65
H
C
C
2.23
H
H
2.65
N
C
C
H
2.02

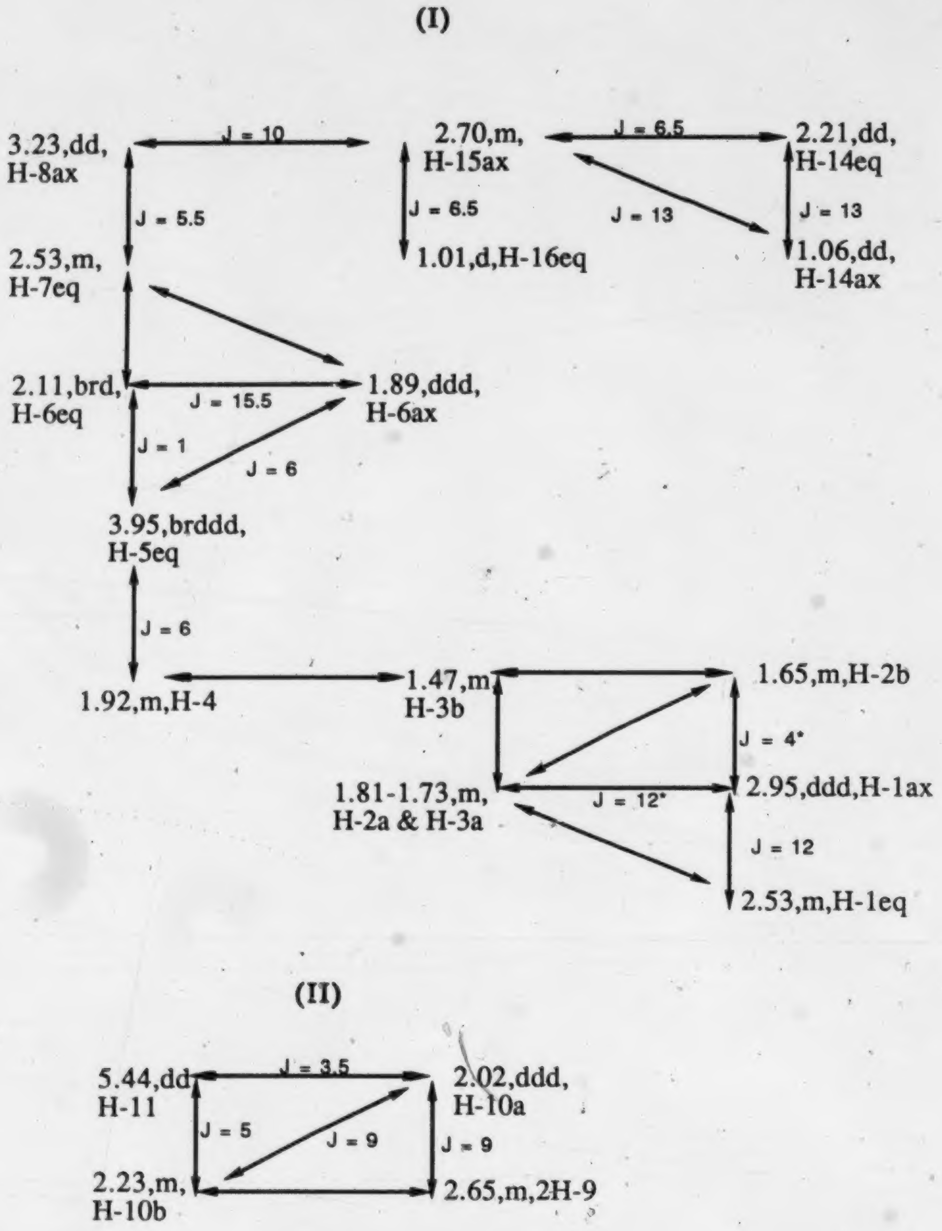
(A)


1.01
H
HO
H
2.7
3.23
H
H
2.21
OH
1.98
H
H
2.11
H
3.95
2.53
H
H
1.89
C
C
H
2.95
N
H
2.53
C

(B)


H_3C
HO
8
15
OH
H
5
N
11
9
H
H

8

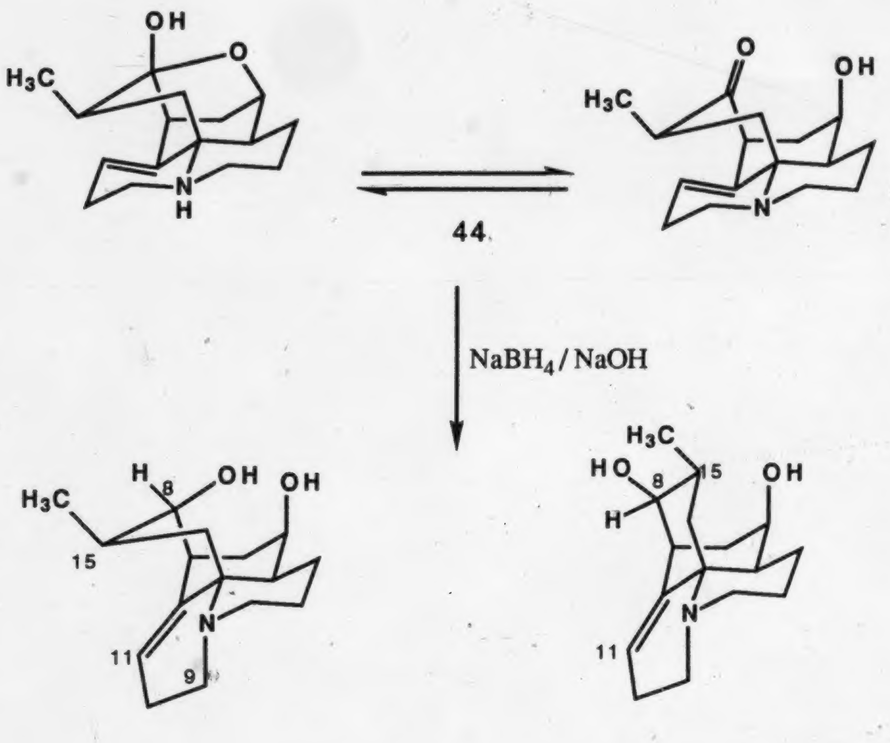

(I)
3.23,dd,
H-8ax
J = 10
2.70,m,
H-15ax
J = 6.5
2.21,dd,
H-14eq
J = 5.5
J = 6.5
J = 13
J = 13
1.01,d,H-16eq
1.06,dd,
H-14ax
2.53,m,
H-7eq
2.11,brd,
H-6eq
J = 15.5
1.89,ddd,
H-6ax
J = 1
J = 6
3.95,brddd,
H-5eq
J = 6
1.92,m,H-4
1.47,m,
H-3b
1.65,m,H-2b
J = 4*
1.81-1.73,m,
H-2a & H-3a
J = 12*
2.95,ddd,H-1ax
J = 12
2.53,m,H-1eq
(II)
5.44,dd,
H-11
J = 3.5
2.02,ddd,
H-10a
J = 5
J = 9
J = 9
2.23,m,
H-10b
2.65,m,2H-9

Scheme 15. ^{1}H-^{1}H chemical shift correlations obtained from the COSY 90 spectrum of lycofoline (**8**).

* coupling constant J may be reversed.

In the ${}^{13}C$ APT experiment, base **8** shows similar carbon-13 signals to those of base **43** except for the carbons of ring D (see table 18). Compound **8** is identical with the known lycofoline[37-40], but the previously reported ${}^{1}H$ nmr data for lycofoline differs somewhat from ours.

To verify our assignments, acrifolinol and lycofoline were prepared from acrifoline (**44**). Acrifoline when reduced with $NaBH_4$ in the presence of NaOH afforded acrifolinol (**43**) and lycofoline (**8**). In the presence of base, acrifoline is partially isomerised at C-15, and is reduced to give the two C-15 epimers, not the C-8 epimers[38]. Synthetic acrifolinol and lycofoline are identical with the bases isolated from natural sources (tlc, ir, ${}^{1}H$ and ${}^{13}C$ nmr spectra).



43

8

Table 18 ^{13}C APT nmr spectra of acrifolinol (43) and lycofoline (8) ($CDCl_3$, 100.6 MHz).

		Chemical shift.in δ.	
C	**mult.**	**43**	**8**
12	s	143.3	143.5
11	d	116.4	116.4
8	d	73.5	79.9
5	d	69.6	68.0
13	s	57.0	56.4
9	t	48.7	48.6
7	d	45.7	47.6
1	t	45.0	45.1
4	d	43.4	45.9
10	t	32.9[a]	32.7[a]
15	d	33.8	31.5
14	t	34.8[a]	32.5[a]
6	t	26.2	26.3
3	t	23.9[b]	24.9[b]
2	t	23.0[b]	23.2[b]
16	q	16.7	20.4

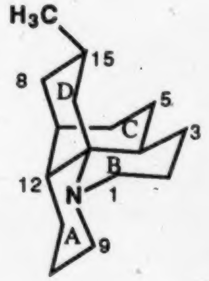
a, signals may be reversed.

b, " " " "

O-acetylannofoline, L17 (47).

The sample of alkaloid L17, isolated from *L. obscurum* by Marion and Manske[11] and obtained from the collection of Marion and Manske's samples, contains a small amount of lycopodine (**6a**), which was easily separated by column chromatography on alumina (see experimental).

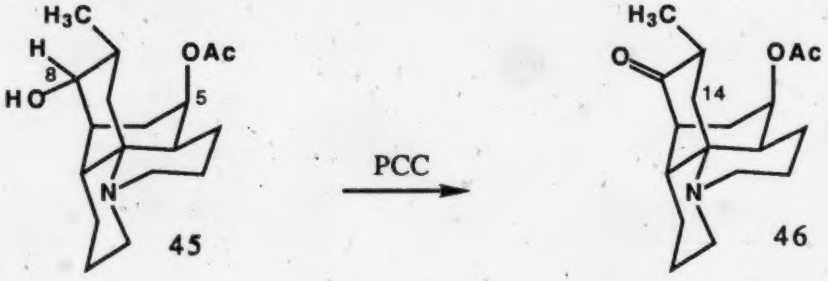
The molecular formula $C_{18}H_{27}NO_3$ (305) for L17 as deduced by elemental analysis was reported by Manske and Marion[11]. We confirmed this molecular formula by hreims. An ester (1739 and 1232 cm^{-1}) and a ketone (1712 cm^{-1}) carbonyl group are evident from the ftir spectrum of L17. The fragmentation pattern of L17 in the hreims conforms to the lycopodane pattern[30]. The base peak at m/z 234, arising from loss of a C_4H_7O unit indicates that the ketone group is in ring D of a lycopodane (**24**) skeleton. The ester group is present as an acetoxyl group as revealed by a peak at m/z 174 (loss of a molecule of acetic acid) and a peak at m/z 146 (loss of acetic acid plus a C_2H_4 unit) from the base peak. The presence of an acetoxyl group is supported by the ^{1}H nmr spectrum (δ 1.92, 3H, s).



24

In the ^{1}H nmr spectrum L17 shows a signal at δ4.83 (apparent quartet, J = 3 Hz) attributed to a hydrogen *geminal* to the acetoxyl group. The coupling pattern for this hydrogen indicates that it is *equatorial.* A secondary methyl group is indicated by a signal at δ1.12 (d, 3H).

That the ketone and acetoxyl group in L17 are substituted at C-8 and C-5, respectively, in a lycopodane skeleton was proved in the following way: α-lofoline **45** was oxidized to dehydro-α-lofoline (**46**) with pyridinium chlorochromate[41]. Dehydro-α-lofoline (**46**) differs from L17 in tlc behavior but their ftir spectra and more especially hreims are very similar. Thus the constitution **46** was assumed for L17 and it remained to determine the stereochemistry. The ^{1}H and ^{13}C nmr spectra of L17 are particularly informative.



The ^{1}H nmr spectrum of L17 shows the hydrogen connectivities in rings C and D which are similar to those in the ^{1}H nmr spectrum of dehydro-α-lofoline (**46**). Signals at δ2.81 (ddq, J = 10,10 Hz) and 2.23 (dd, J = 14,10 Hz) are attributed to *vicinal axial* hydrogens, H-15 and H-14, respectively. The chemical shift and coupling pattern of H-15 deduced from decoupling experiments (table 19) shows that it is α to the C-8 ketone group. Decoupling experiments show that H-7 (δ2.26) is coupled to the methylene hydrogens at C-6 (δ2.09 and *ca* 1.85), which are further coupled to the carbinyl hydrogen (δ4.84).

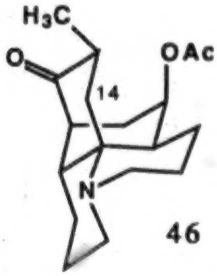
Table 19 Deco[illegible]eriments in the ^{1}H nmr spectrum of acetylannofoline (47) (C[illegible]Hz).

Chemical shift in δ, mult., J in Hz.

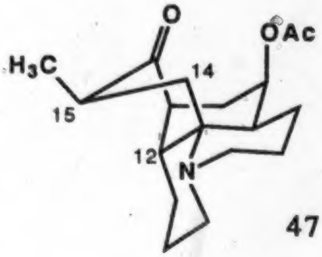
Signal irradiated	Observed change	
4.84,app.q,3(H-5eq)	2.34,dt,12.5,3(H-4ax)	dd,12.5,3
	2.09,dt,15,3(H-6eq)	dd,15,3
	1.85,m(H-6ax)	pert.
2.81,ddq,10,10,6(H-15ax)	2.23,dd,14,10(H-14ax)	d,14
	1.27,m(H-14eq)	pert.
2.26,ddd,5,3,3(H-7eq)	2.09,dt,15,3(H-6eq)	dd,15,3
	2.26,ddd,5,3,3(H-7eq)	pert.
	1.85,m(H-6ax)	pert.
2.09,dt,15,3(H-6eq)	4.84,app.q,3(H-5eq)	t,3
	1.85,m(H-6ax)	pert.
1.12,d,6(H-16)	2.81,ddq,10,10,6	dd,10,10

In the ^{1}H nmr spectrum of dehydro-α-lofoline H-14ax resonates at δ3.07 (dd, J = 14,10 Hz), downfield from the signal attributed to H-14ax (δ2.23) in the ^{1}H nmr spectrum of L17. Comparison of the ^{13}C nmr spectra of L17 and compound **46** show their structure similarities (table 20). The carbon signals of L17 are similar to those of dehydro-α–lofoline (**46**) (within Δ 0.1-0.4) except for the carbons of ring D and vicinity (carbon 8, 13, 14, 16 and 18). C-14 (δ31.5) is particularly further upfield in L17 than in **46** (δ39.9).

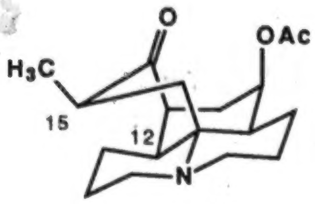
At this stage it is clear that alkaloid L17 is isomeric with dehydro-α-lofoline (**46**) at either, or both of, C-12 and C-15. Structure **47** is epimer at C-15 and H-15 has the required *vicinal antiperiplanar* hydrogens. Structure **48** is isomeric with **46** at both C-12 and C-15, but requires an unfavorable boat conformation of ring D to maintain the required H-14, H-15 coupling. Structure **49** differs from **46** only at C-12.



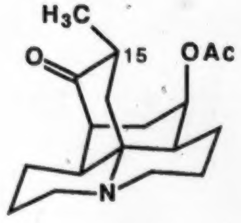
46



47

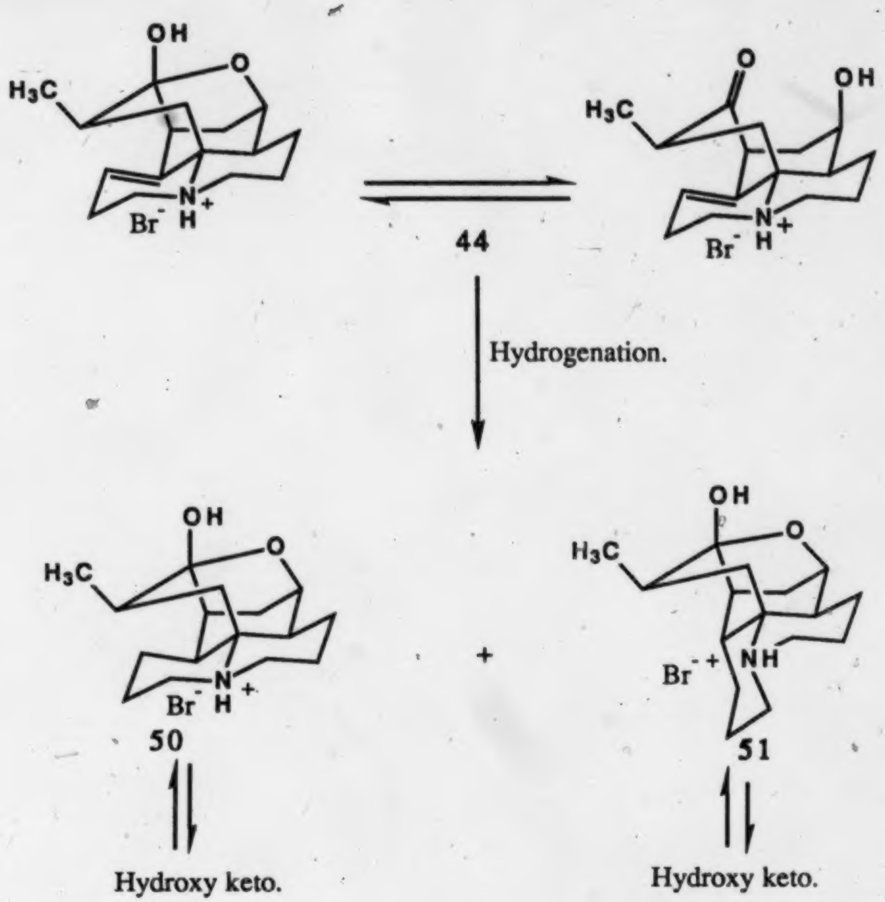


48

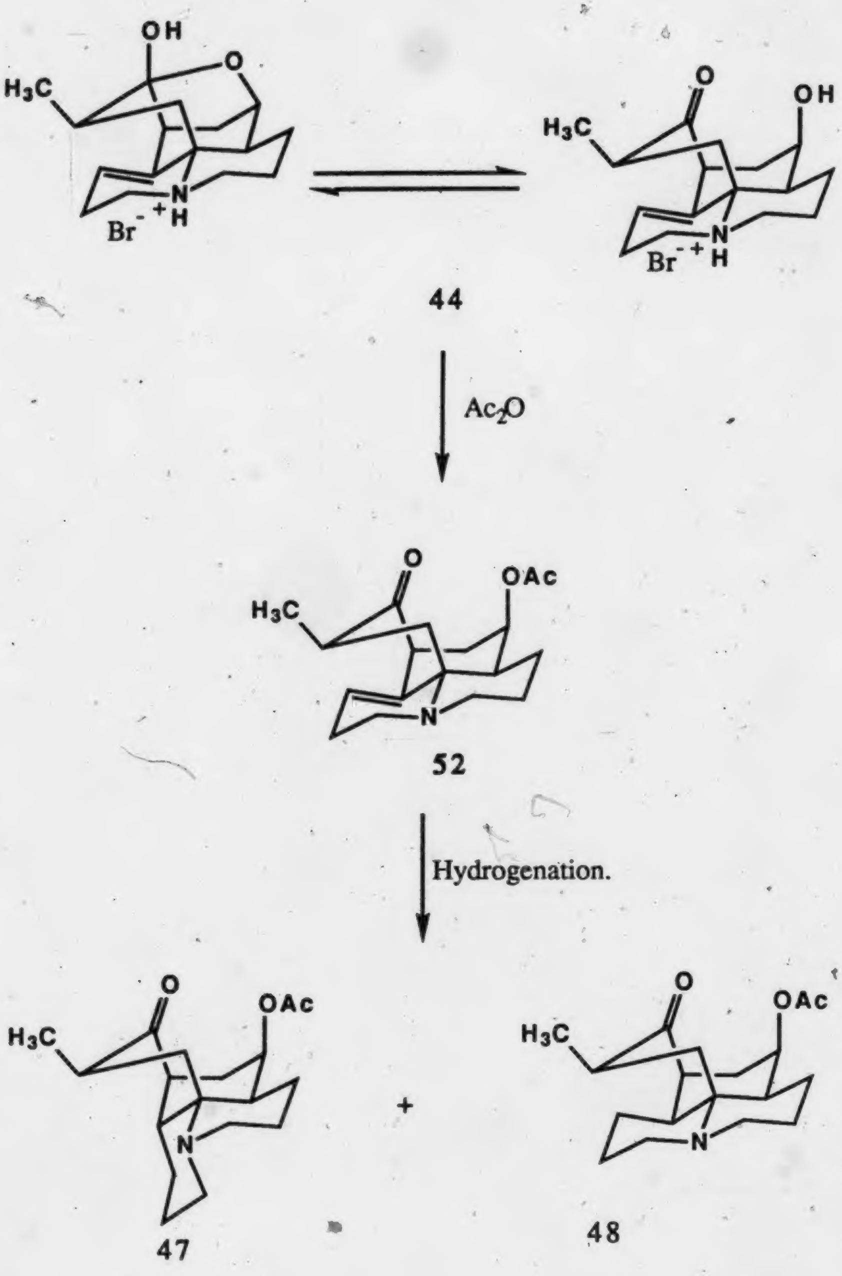


49

It is known that catalytic hydrogenation of acrifoline hydrobromide (**44**) affords dihydroacrifoline (**50**) along with small amounts of annofoline (**51**)[42]. Dihydroacrifoline and annofoline are diastereomeric with the configuration at C-15 opposite to that of dehydro-α-lofoline (**46**). Thus acrifoline was acetylated to give O-acetylacrifoline (**52**)[36], which was subjected to catalytic hydrogenation with Adams' catalyst. Dihydro-O-acetylacrifoline (**48**) was obtained along with small amount of acetylannofoline (**47**). Dihydro-O-acetylacrifoline shows a fragmentation pattern in its hreims similar to that of


OH
O
H3C
N+
Br- H
44
O
OH
H3C
N+
Br- H+
Hydrogenation.
OH
O
H3C
N+
Br- H+
50
Hydroxy keto.
OH
O
H3C
Br- + NH
51
Hydroxy keto.

L17 and dehydro-α-lofoline (**46**) but differs a great deal in tlc behavior, ftir, ^{1}H and ^{13}C nmr (see table 20). Acetylannofoline (**47**) on the other hand shows similar tlc behavior, hreims and a ^{1}H nmr spectrum identical with that of L17. The sample of acetylannofoline was not large enough to obtain additional spectral data. A larger sample was prepared in the following way: dehydro-α-lofoline (**46**) was epimerized in dilute base to annofoline (**51**). Upon acetylation annofoline afforded O-acetylannofoline (**47**) almost quantitatively. Alkaloid L17 is identical with acetylannofoline in all respects (tlc, ftir, hreims, ^{1}H and ^{13}C nmr).


OH
O
H$_3$C
N
Br$^-$ $^+$H
O
OH
H$_3$C
N
Br$^-$ $^+$H
44
Ac$_2$O
O
OAc
H$_3$C
N
52
Hydrogenation.
O
OAc
H$_3$C
N
47
+
O
OAc
H$_3$C
N
48

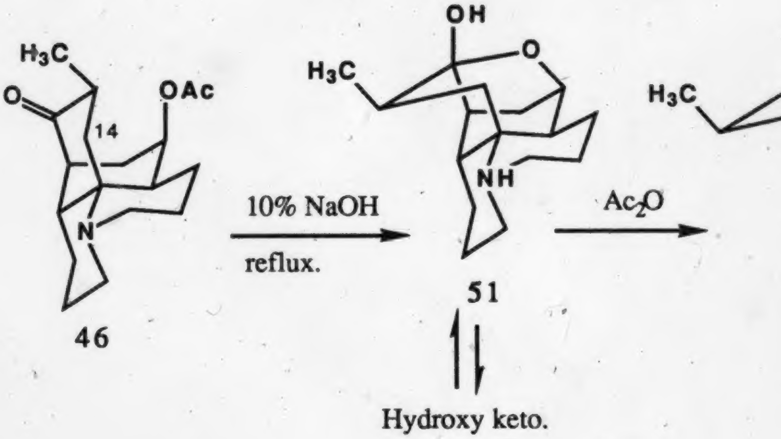

H₃C
O
14
OAc
N
46
10% NaOH
reflux.
OH
O
H₃C
NH
51
Hydroxy keto.
Ac₂O
O
OAc
H₃C
N
47

Table 20 ^{13}C nmr spectra of α-lofoline (45), dehydro-α-lofoline (46), O-acetylannofoline (47) and dihydro-O-acetylacrifoline (48) ($CDCl_3$, 100.6 MHz).

		Chemical shift in δ.			
C	mult.	45[76]	46	47	48
1	t	46.9	46.9	46.6	49.0
2	t	18.1	19.4	19.0	23.0
3	t	20.3	23.5	23.3	24.4
4	d	31.1	31.3	38.4[a]	40.3
5	d	70.2	72.1	73.0	72.6
6	t	29.3	30.1	29.8	37.9
7	d	42.8	47.9	46.5	46.4
8	d	75.2	218.0(s)	216.8(s)	216.1(s)
9	t	47.2	47.8	48.1	49.0
10	t	26.4	26.0[b]	26.0[a]	25.5[a]
11	t	24.2	23.5[b]	23.4[a]	25.3[a]
12	d	37.4	40.3[a]	40.1	47.3
13	s	54.6	54.0	54.7	55.6
14	t	38.3	39.9	31.5	28.0
15	d	27.5	39.3[a]	39.2[a]	50.0
16	q	23.0	22.6	21.2	21.3
$\underline{C}O$	s	170.2	169.8	170.0	170.1
$\underline{C}H_3CO$	q	21.4	20.9	14.6	16.2

III Mentha arvensis.

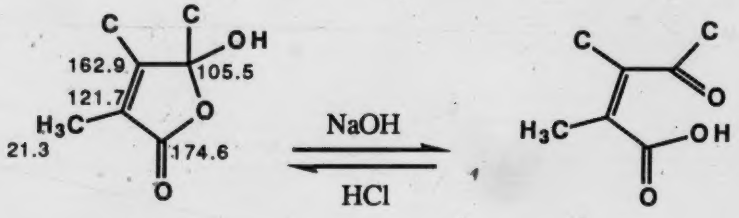
Mentha arvensis (Labiatae), commonly called wild mint, is an indigenous Canadian plant used in Canadian folk-medicine to treat a variety of illnesses including heart diseases[13]. Another Labiatae plant, *Coleus forskohlii* Briq, a member of the same phylogenic family as mint, has been reported to contain forskolin (5)[14]. Forskolin lowers blood pressure[15] and this property has stimulated interest in its potential use as a therapeutic agent. The close relationship between *C. forskohlii* and *M. arvensis* and the several reports that wild mint had been used to treat heart disease prompted us to undertake a chemical investigation of the metabolites of *M. arvensis.*

In our early studies, *M. arvensis* was extracted using a procedure developed by Inamdar and coworkers for assay of forskolin in *C. forskohlii* [15] (see scheme 25, Experimental). The *M. arvensis* extracts were screened for the presence of forskolin using thin-layer chromatography as the method of analysis. The thin-layer chromatograms of the crude extracts were obtained and compared with that of an authentic sample of forskolin. No isolable nor tlc detectable forskolin was present in the extracts. Further chemical investigation of the *M.* arvensis extracts led to the separation and isolation of the metabolites described below.

Lactone (54).

Concentration of the Skellysolve B extract gave a precipitate, which was separated from the mother liquors and recrystallized from Skellysolve B - ethyl acetate to afford compound **54** as colourless needles, mp 190-191°C .

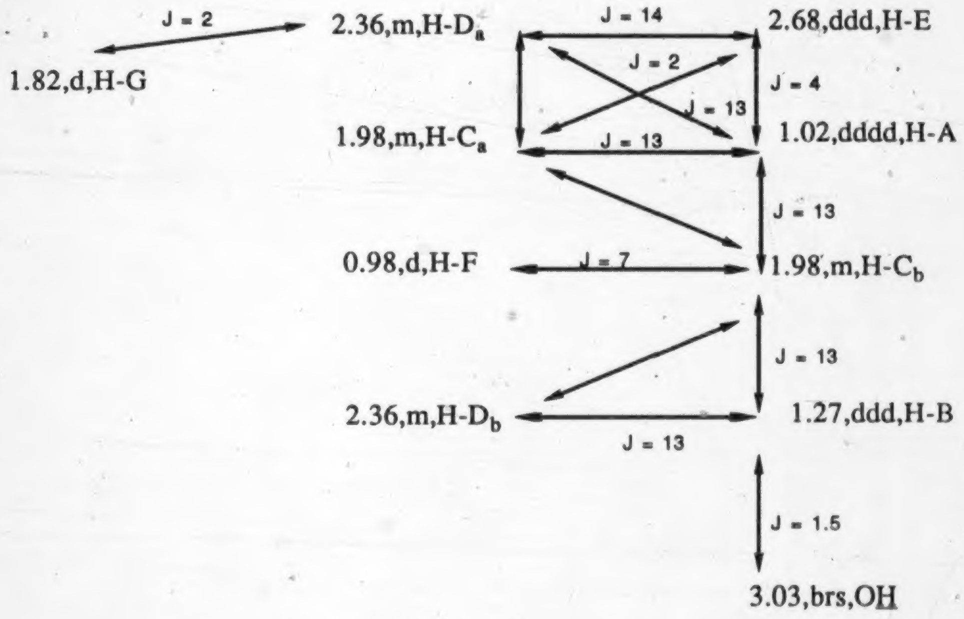
Compound **54** is optically active ($[\alpha]_D = -60.7$) and has a molecular formula, $C_{10}H_{14}O_3$ (182.0946) as indicated by hreims. Its ftir spectrum displays strong, sharp absorption bands indicative of hydroxyl (s) (3345 cm^{-1}) and carbonyl (1745 cm^{-1}) groups. The ^{13}C nmr spectrum reveals that the carbonyl (δ 174.6) group in **54** is present as a lactone or ester. The ^{1}H and ^{13}C nmr spectra of **54** also reveal the presence of secondary (^{1}H nmr : δ 0.98, d, J = 7 Hz; ^{13}C nmr : δ 7.9) and alkenic (^{1}H nmr : δ 1.8, d, J = 2 Hz; ^{13}C nmr : δ 21.3) methyl groups. Compound **54** displays a uv absorption, λ_{max} 215 (4.39) nm. Addition of NaOH causes a reversible (upon addition of HCl) bathochromic shift to λ_{max} 260 (3.7) nm. This information together with the ftir data suggest the presence of an α, β-unsaturated γ lactol, partial structure A. The failure of compound **54** to undergo methylation with diazomethane indicates that partial structure A exists entirely in the lactol form A. Support for this partial structure is available from the ^{13}C nmr spectrum, which displays signals at δ 174.6 (s), 162.9 (s), 121.7 (s), 105.5 (s), and 21.3 (q), each assigned as shown in the partial structure A.



(A)

Partial structure A accounts for three of the four unsaturation equivalents indicated by the molecular formula, thus, the monoterpene **54** is bicyclic. The unassigned carbon-13 signals, three methylene (δ 47.1, 36.1, 25.2) groups and one methine (δ 30.5) group, allow us to conclude that the second ring is a cyclohexane.

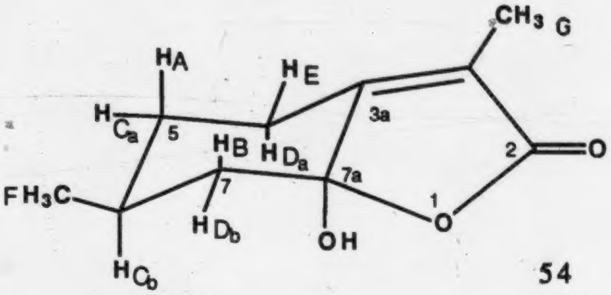
Extensive spin decoupling experiments in the ^{1}H nmr (table 21) revealed the constitution (scheme 16) of the cyclohexane ring. The alkenic methyl group (δ 1.82) is homoallylically coupled to hydrogen D$_a$ (δ 2.36). Upon irradiation of the multiplet (δ 2.36) due to the hydrogens D$_a$ and D$_b$, the alkenic methyl signal collapses to a singlet, while this multiplet (δ 2.36) is perturbed when the alkenic methyl signal is irradiated. The *geminal* partner to H-D$_a$ is H-E (δ 2.68, ddd, J = 14,4,2 Hz). The coupling pattern of H-E indicates that it is oriented *equatorially*. Both H-E and H-D$_a$ are coupled to methylene hydrogens A (δ 1.02) and C$_a$ (δ 1.98), which are in turn coupled to a methine hydrogen C$_b$ (δ 1.98). The methine hydrogen C$_b$ is also coupled to the methyl group (δ 0.98) and to methylene hydrogens B (δ 1.27) and D$_b$ (δ 2.36). The Hydrogen B (δ 1.27) is further coupled to the hydroxyl hydrogen (δ 3.03). Structure **54**, in which the cyclohexane ring adopts a chair conformation, is consistent with these data.


J = 2
2.36,m,H-Da
J = 14
2.68,ddd,H-E
1.82,d,H-G
J = 2
J = 4
J = 13
1.98,m,H-Ca
J = 13
1.02,dddd,H-A
J = 13
0.98,d,H-F
J = 7
1.98,m,H-Cb
J = 13
2.36,m,H-Db
1.27,ddd,H-B
J = 13
J = 1.5
3.03,brs,OH

Scheme 16. ^{1}H nmr coupling pattern of lactone (**54**) (CDCl$_3$, 400 MHz)

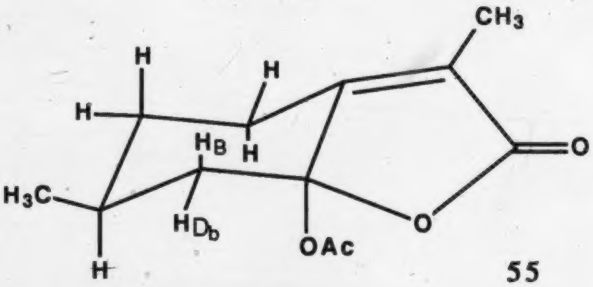
Table 21 Decoupling experiments in the ^1H nmr spectrum of lactone (54) ($CDCl_3$, 400 MHz).

Signal irradiated	Chemical shift in δ, mult., J in Hz. Observed change	
0.98,d,7 (H-9) & 1.02,dddd,13,13,13,4 (H-5ax)	1.98,m (H-5eq,H-6ax)	pert.
	2.36,m (H-4ax)	pert.
	2.68,ddd,14,4,2 (H-4eq)	dd,14,2
1.02,dddd,13,13,13,4 (H-5ax)	1.98,m (H-5eq,H-6ax)	pert.
	2.36,m (H-4ax)	pert
	2.68,ddd,14,4,2 (H-4eq)	dd,14,2
1.27,ddd,13,13,1.5 (H-7ax)	1.98,m (H-6ax)	pert.
	2.36,m (H-7eq)	pert.
1.82,d,2 (H-8)	2.36,m (H-4ax)	pert.
1.98,m (H-5eq,H-6ax)	0.98,d (H-9)	s
	1.02,dddd,13,13,13,4 (H-5ax)	dd,13,4
	1.27,ddd,13,13,1.5 (H-7ax)	brdd,13,13
	2.36,m (H-7eq)	pert.
	2.68,ddd,14,4,2 (H-4eq)	dd,14,4
2.36,m (H-4ax,H-7eq)	1.02,dddd,13,13,13,4 (H-5ax)	pert.
	1.27,brddd,13,13,1.5 (H-7ax)	brd,13
	1.82,d,2 (H-8)	s
	1.98,m (H-5eq,H-6ax)	pert.
	2.68,ddd,14,4,2 (H-4eq)	dd,4,2
2.68,ddd,14,4,2 (H-4eq)	1.02,dddd,13,13,13,4 (H-5ax)	ddd,13,13,13
	1.98,m (H-5eq)	pert.
	2.36,m (H-4ax)	pert.
3.03,brs,OH	1.27,brddd,13,13,1.5 (H-7ax)	brd,13,13



54

Upon acetylation, lactone **54** afforded a monoacetate **55**, $C_{12}H_{16}O_4$ (224.1048), as shown by hreims. The ftir spectrum of the monoacetate **55** shows the absence of a hydroxyl group, and presence of a carbonyl (1700 cm^{-1}) group.

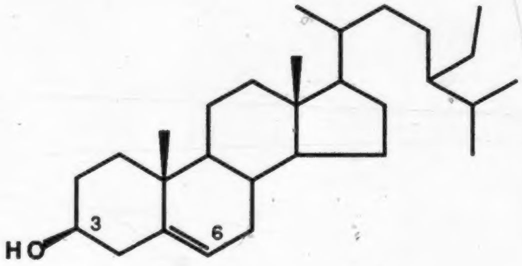


55

In the ^1H nmr spectrum of monoacetate **55**, the methylene hydrogens B (δ 1.14) and D_b (δ 2.69), previously observed at δ 1.27 and 2.36, respectively, in the ^1H nmr spectrum of lactone **54** are consistent with an *axially* oriented acetoxyl group. The hydrogen H-B is in the shielding zone, while H-D_b is in the deshielding zone of the acetoxyl group. The hydroxyl and methyl group in lactone **54** are, therefore, *trans* to one another.

Lactone **54** is identical with the compound previously isolated from peppermint oil, or synthesised from methofuran (by autoxidation)[43], and from d-pulegone[44] (mp, ir, optical rotation, ^{1}H nmr spectrum). Its enantiomer has been isolated from *Bursera graveolens* (H.B.K.) Tr. et Pl. var *villosula* Cuatr[45].

Fatty acids, a mixture of monooxgenated triterpenoids, and a steroid identified as β-sitosterol (**56**) were also separated and identified from the Skellysolve B extract *M. arvensis*. β-Sitosterol was identified by comparison (ir, hreims, and ^{1}H nmr)[46-48] with an authentic sample.



56

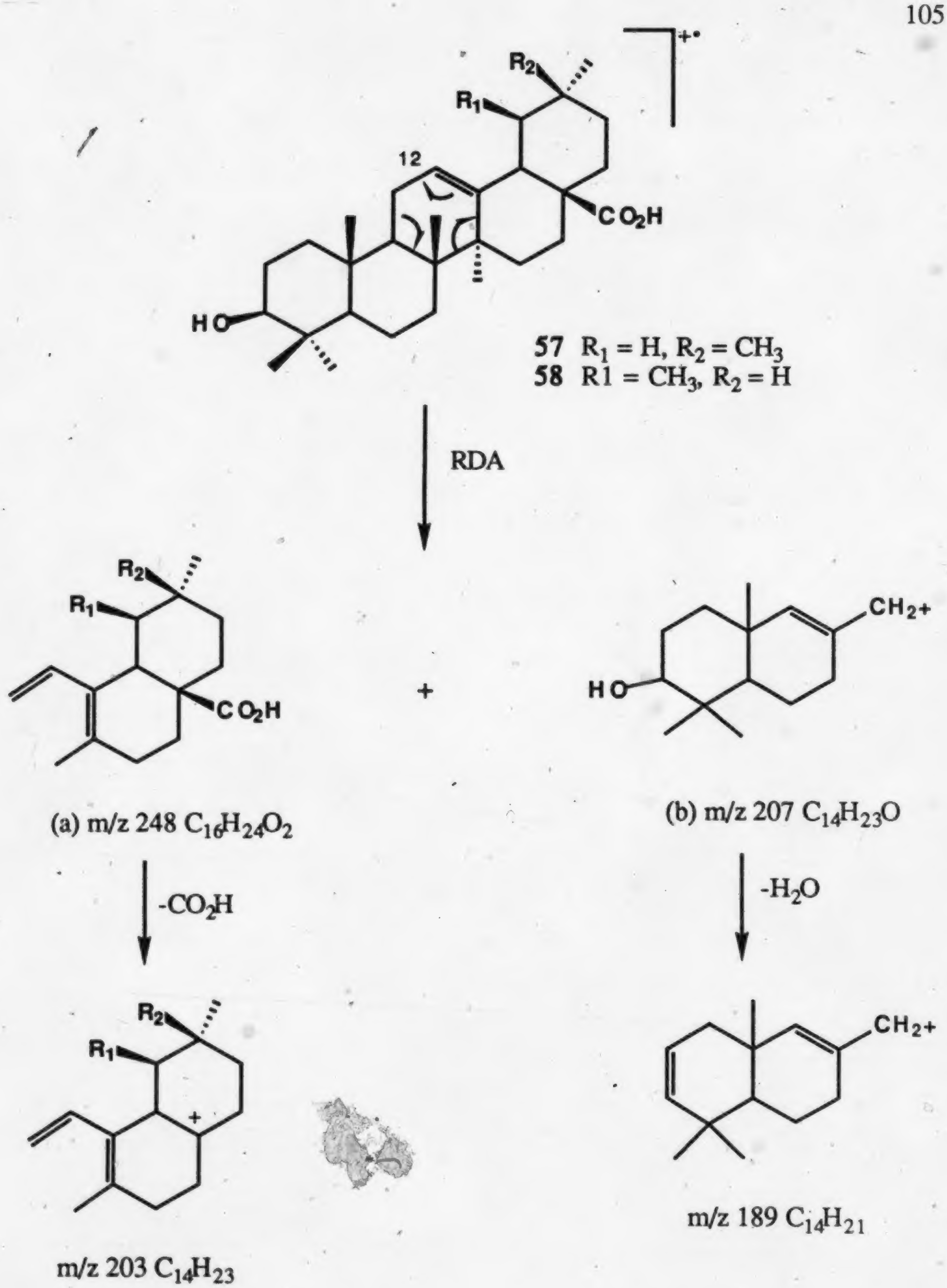
Oleanolic (57), ursolic (58), and pomolic (65) acid.

The principal metabolites of the chloroform extract of *M. arvensis* are triterpene acids These metabolites were also detected in the Skellysolve B extract but in smaller amount. The major component of the triterpene acid fraction, which on tlc appears as a single spot and is eluted as one component by flash column chromatography, was identified as a mixture of two closely related, isomeric triterpene acids, compounds, **57** and **58**. The mixture could not be separated by either column or thin-layer chromatography using a variety of solvent systems. It is sparingly soluble in chloroform and in methanol, but dissolves in a mixture of both solvents. Crystallization of the component from chloroform or methanol affords the mixture **A** as an offwhite powder.

The molecular formula, $C_{30}H_{48}O_3$ (456), of the mixture **A** was deduced by hreims and cims. The ftir spectrum of the mixture **A** displays a weak hydroxyl ($3400\,cm^{-1}$) and a strong carbonyl ($1685\,cm^{-1}$) absorption. The presence of these functionalities was confirmed in the hreims of **A**, since peaks at m/z 438 and 411 arise from loss of a molecule of water and a CO_2H unit, respectively, from the parent ion. The absence of a broad hydroxyl absorption at about $3600\text{-}2500\,cm^{-1}$ in the ftir spectrum, generally observed in the ir of carboxylic acids, and the failure of the corresponding methyl esters to undergo saponification or reduction with $LiAlH_4$ indicates that the carbonyl moiety in each triterpene is hindered.

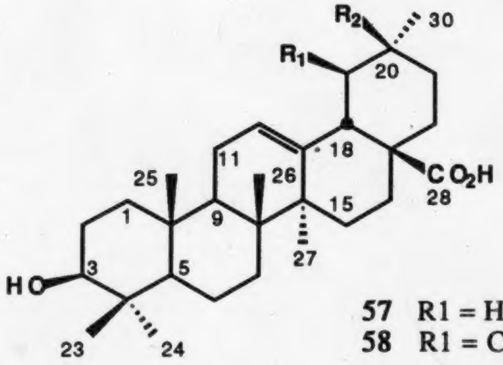
The base peak in the hreims of the mixture **A** appears at m/z 248, $C_{16}H_{24}O_2$, an ion commonly observed in the mass spectra of Δ^{12} oleanene and ursene triterpene acids[49]. This ion is consistent with fragment (**a**) arising from a retro Diels-Alder (RDA) fragmentation as shown in scheme 17. The peaks at m/z 203 and 189 arise from loss of a CO_2H unit from fragment (**a**) and loss of RH from fragment (**b**), respectively.

The ^1H nmr spectrum of the mixture A displays a pair of olefinic (δ 5.29 and 5.25, each a t, J = 3.5 Hz) and carbinyl (δ 3.23 and δ 3.20, each a brs, $W_{1/2}$ = 18 Hz) hydrogens. As well, there are two well resolved signals at *ca* δ 2.82 (brdd, J = 14,4.5 Hz) and 2.20 (brd, J = 12 Hz), each is assigned to an H-18β of Δ^{12} oleanene and ursene triterpenes, respectively. This hydrogen is reported to resonate at a similar chemical shift in the ^1H nmr of these compounds[50].

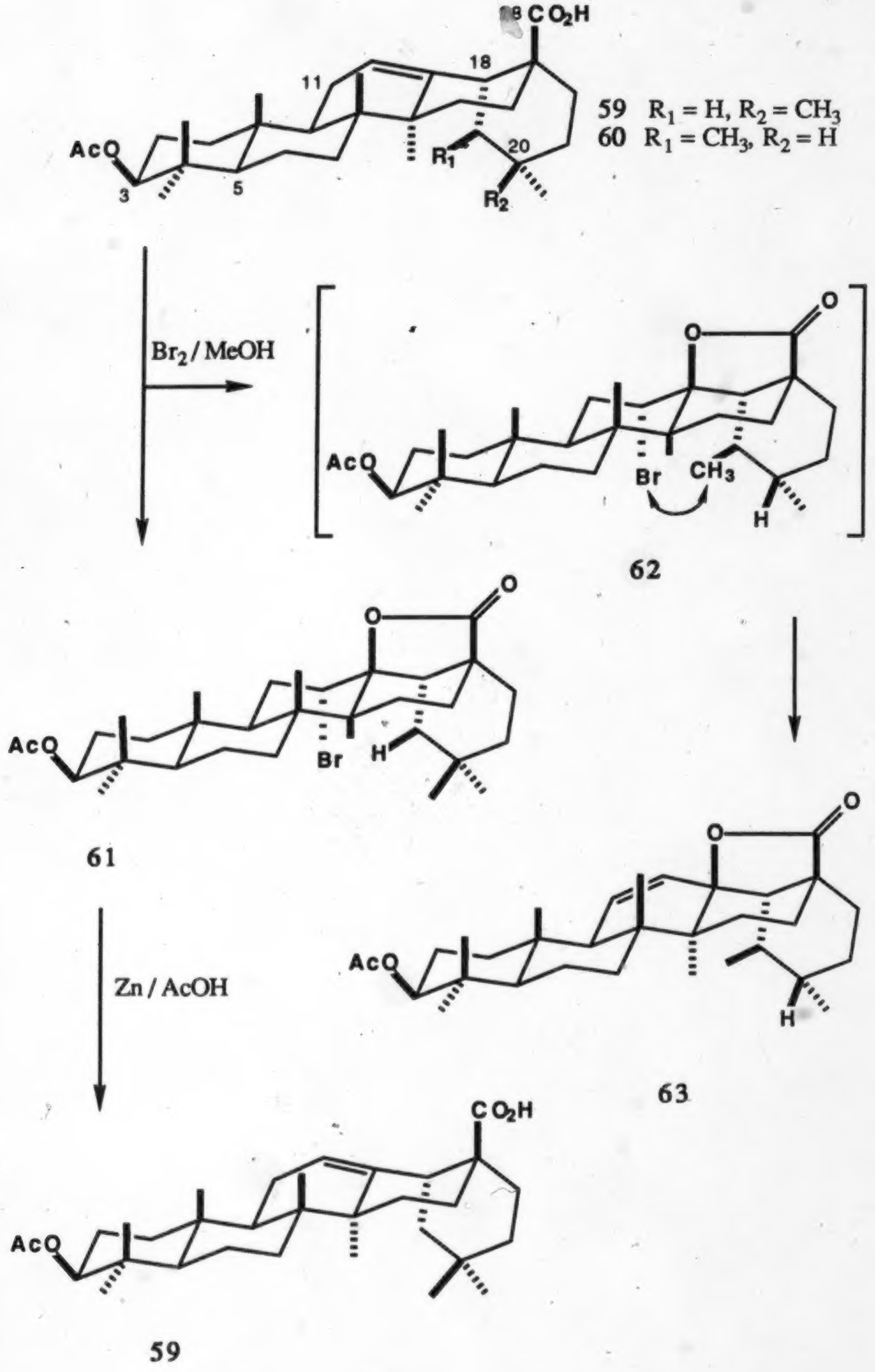

+•
R2
R1
12
CO2H
HO
57 R1 = H, R2 = CH3
58 R1 = CH3, R2 = H
RDA
R2
R1
CO2H
+
CH2+
HO
(a) m/z 248 C16H24O2
(b) m/z 207 C14H23O
-CO2H
-H2O
R2
R1
+
CH2+
m/z 203 C14H23
m/z 189 C14H21

Scheme 17. Fragmentation in the hreims of Δ^{12} oleanene and ursene triterpene acids.)

The triterpenes of the mixture **A** were separated and identified as oleanolic (**57**) and ursolic (**58**) acid in the following way: the mixture **A** was converted to a mixture of acetates, compounds **59** and **60**. The mixture of acetates was treated with a solution of bromine at room temperature[51]. The high Rf component was identified as bromolactone **61**, $C_{32}H_{49}O_4Br$ (577 as shown by cims and hreims). The signal at δ 4.28 (dd, J = 4,1.5 Hz, 1H) in the ^1H nmr spectrum of lactone **61** is assigned to an *equatorial* hydrogen, *geminal* to an *axial* bromine atom. The ftir spectrum of **61** shows a carbonyl absorption at 1774 cm^{-1}, which is characteristic of a γ lactone[20]. Lactone **61** afforded acetate acid **59** upon exposure to zinc dust in acetic acid. The ^1H nmr spectrum of **59** displays the H-18β allylic hydrogen at δ 2.78 (ddd, J = 14,4.5,1 Hz) and seven angular methyl groups. These data are consistent with that reported for 3β-acetylolenolic acid (**60**)[52] (see table 22 for comparison of methyl group signals in the ^1H nmr spectrum).

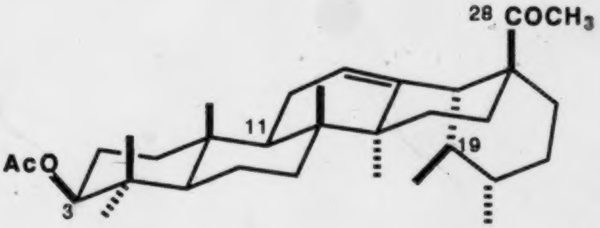


57 R1 = H, $R_2 = CH_3$
58 R1 = CH_3, R_2 = H


CO₂H
11
18
AcO
3
5
R₁
20
R₂
59 R₁ = H, R₂ = CH₃
60 R₁ = CH₃, R₂ = H
Br₂ / MeOH
O
O
AcO
Br
CH₃
H
62
O
O
AcO
Br
H
61
Zn / AcOH
O
O
AcO
H
63
CO₂H
AcO
59

The second least polar component from the bromolactonisation reaction mixture was tentatively identified as lactone **63**, $C_{32}H_{48}O_4$ (496.3557,as shown by hreims). The ftir spectrum of **63** displays γ lactone (1755 cm^{-1}) and acetoxyl (1734 cm^{-1}) carbonyl groups. Its ^1H nmr spectrum shows two *vicinal, cis* olefinic hydrogens (δ5.94, dd, J = 10,1 Hz and 5.56, dd, J = 10,2.5 Hz). The presence of five angular methyl (δ 1.16, 1.06, 0.94, 0.87, 0.86) and two secondary methyl (δ 1.0, d, J = 6 Hz and 0.94, perturbed* d, J = 6 Hz) groups in the ^1H nmr spectrum of **63** suggest that it is derived from acetyl ursolic acid (**60**). In acetyl ursolic acid, bromolactonisation is accompanied by debromination, presumably to relieve steric crowding in intermediate **62** between the C-12, *axial* bromine atom and the C-19β *equatorial* methyl group.

Mixture A was esterified (CH_2N_2) and acetylated (Ac_2O, pyr). Repeated fractional crystallization (methanol) of the acetate ester mixture led to the isolation of a single ester acetate, compound **64**. The ^1H nmr spectrum of the ester acetate **64** shows signals assigned to H-18β (δ2.23, d, J = 11 Hz), five angular methyl (δ1.08, 0.95, 0.87, 0.86, 0.75), and two secondary methyl (δ1.00, d, J = 6 Hz and 0.94, Perturbed d, J = 6 Hz) groups. These ^1H nmr data allowed us to identify compound **64** as methyl 3β-acetylursolate[53] (see table 22 for comparison of methyl group signals in the ^1H nmr spectrum).



64

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* Second order perturbation effect on secodary methyl groups in the ursene series has been discussed by Cheung[50].

Table 22 Comparison of methyl group signals in the ^1H nmr spectra of 3β-acetyloleanolic acid (60) and methyl 3β-acetylursolate (64) with literature data.

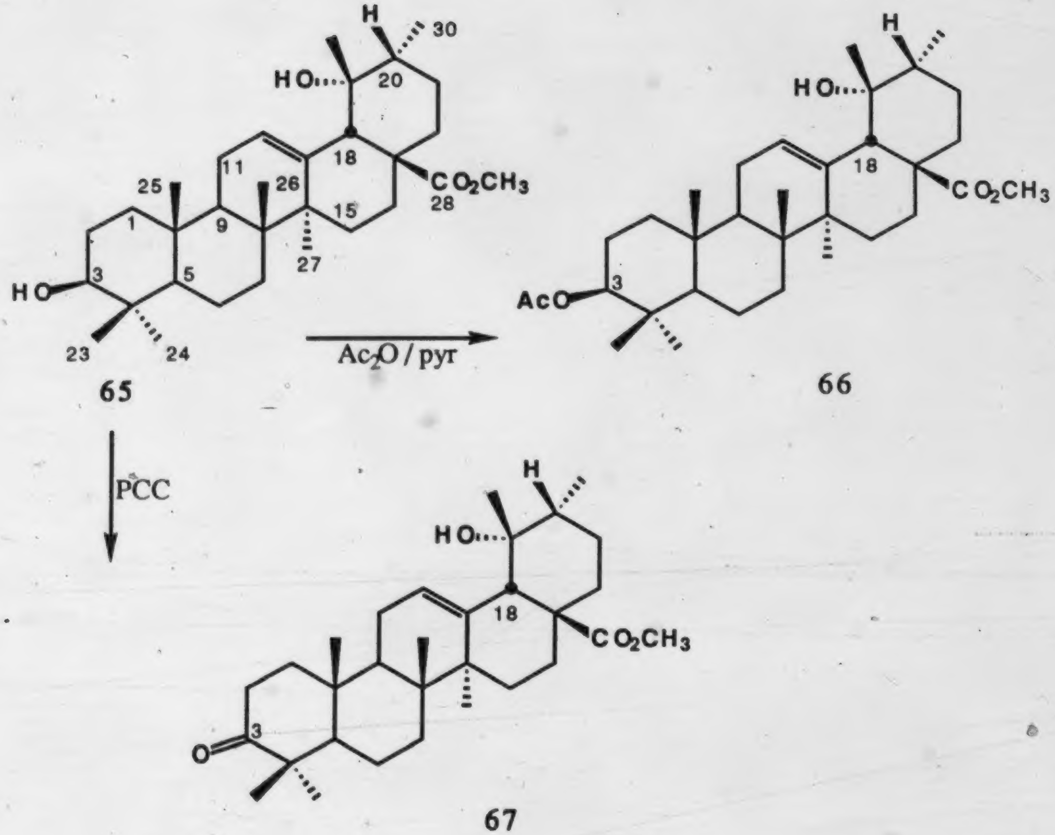
	Chemical shift in δ.	
H	**60(lit)[52]**	**64(lit)[53]**
23(s)	0.86[a](0.87)	0.86[a](0.86)
24(s)	0.87[a](0.87)	0.87[a](0.86)
25(s)	0.91[b](0.88-0.98)	0.95[b](0.95)
26(s)	0.76(0.76)	0.75(0.75)
27(s)	1.14(1.15)	1.08(1.08)
29(s)	0.93[b](0.88-0.98)	0.87(d)(m)
30(s)	0.94[b](0.88-0.98)	0.94[b](d)(m)

a, signals may be reversed.

b, " " " "

A third triterpene acid was isolated from the crude chloroform fraction and characterised as its methyl ester derivative compound **65**. The ftir spectrum of **65** displays absorptions attributable to hydroxyl(s) (3610, 3540, 3420 cm^{-1}) and methyl ester carbonyl (1718 cm^{-1}).

Triterpene **65** resembles oleanolic (**57**) and ursolic acid (**58**) in the ^1H nmr and hreims : the ^1H nmr spectrum of compound **65** indicates an olefinic hydrogen (δ5.36), a carbinyl hydrogen (δ 3.22), as well as an allylic hydrogen (δ 2.60, brs, $W_{1/2} = 4$ Hz), which is assigned to H-18β in the oleanene or ursene class of triterpenes. The molecular formula, $C_{31}H_{50}O_4$ (486.3701) of triterpene **65** as shown by hreims and cims differs from that of methyl oleanolate and methyl ursolate by one oxygen. However, the fragmentation pattern of each ester is similar. Chemical derivatisation and detailed examination of ^{1}H nmr spectrum of triterpene **65** revealed that the fourth oxygen in the molecular formula is present as a tertiary hydroxyl group. Upon acetylation (pyr, Ac_2O, room temperature), **65** afforded a monoacetate methyl ester **66**. Its ftir spectrum displays hydroxyl (3530 cm^{-1}) as well as methyl ester acetoxyl carbonyl (1723 cm^{-1}). When **65** is oxidised (PCC), a ketoester **67** is obtained. The ftir spectrum of **67** displays hydroxyl (3530 cm^{-1}), as well as methyl ester (1721 cm^{-1}), and ketone (1704 cm^{-1}) carbonyls. Furthermore, in the ^{1}H nmr spectra of each of **65** and its derivatives **66** and **67** there is an allylic methine hydrogen appearing as a singlet which may be attributed to H-18β, as well as six angular methyl groups and a secondary methyl group. This information is consistent with substituted methyl ursolate, where C-19 is fully substituted and bears the tertiary hydroxyl group. The presence of a hydroxyl group at C-19 is consistent with the downfield shift of H-18β and the angular C-29 methyl group in the ^{1}H nmr spectra of **65**, **66**, and **67** compared with the ^{1}H nmr spectra of ursolic acid **57** and its acetyl ester derivative **64** (see table 21 for comparison). In addition, the C-27 angular methyl group is shifted downfield, while the C-26 angular methyl group is shifted upfield in the ^{1}H nmr spectrum of triterpene **66** when compared with the ^{1}H nmr spectrum of triterpene **64**. This indicates that the C-27 and the C-26 methyl groups are in the deshielding and shielding zones of the hydroxyl group, respectively. The hydroxyl group is thus α oriented. The spectral data obtained for triterpene **65** is identical with that reported for methyl pomolate (3β, 19α-dihydroxy-urs-12-en-28-oate) (ftir, hreims, and ^{1}H nmr spectrum). Pomolic acid has previously been isolated from Japanese *M. arvensis* var[54] and from *Micromerias benthani* Webb et Berth (Labiatae)[55].


HO
CO$_2$CH$_3$
H
30
20
18
11
26
25
1
9
15
28
27
3
5
23
24
Ac_2O / pyr
PCC
AcO
O

65

66

67

Table 23 ^{1}H nmr spectra of methyl 3β-acetylursolate (64) and methyl 3β-acetylpomolate (66).

H	Chemical shift in δ, mult. J in Hz. 64	 66
3	4.50,brdd,10,5	4.50,brdd,9,7
12	5.25,t,4	5.36,t,3
18	2.23,brd,11	2.60,brs,$W_{1/2} = 4$
23	0.86,s	0.86,s
24	0.87,s	0.87,s
25	0.95,s	0.93,s
26	0.75,s	0.68,s
27	1.08,s	1.22,s
29	0.87,d,6	0.95,d,7
30	0.98,d,6	0.95,d,7
$C\underline{H}_3CO_2$-	2.05	2.05
$C\underline{H}_3O_2$-	3.60	3.60

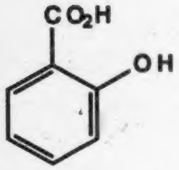
IV. *Gerarium viscosissimum* and *Eriogonum umbellatum*

The use of plants as herbal remedies is a popular traditional practice in many parts of the world, especially in the under developed countries. In North America, the native Indians use indigenous plants to treat a wide variety of illnesses[13b]. A number of these indigenous plants have been used for treatment of infectious diseases such as inflammation, sores, colds, coughs etc. A chemical investigation of the metabolites of two of these plants is described in this section.

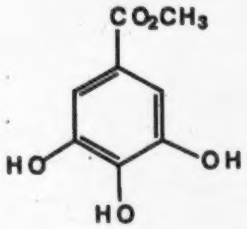
In our preliminary studies, dry, ground plant was extracted with methanol using a Soxhlet apparatus. The crude methanol extract was partitioned into Skellysolve B, ether, and ethyl acetate solubles as shown in scheme 27 (Section D1, Experimental). The various extracts were screened for antibacterial activity using the modified Kirby-Bauer test as described in Section D2 of the Experimental. The results of the biological tests with the extracts of *Geranium viscosissimum* and *Eriogonum umbellatum* are shown in table 24. The crude ether extract of *G. viscosissimum* and the crude ether and ethyl acetate extracts of *E. umbellatum* exhibit significant antibacterial activities against a variety of bacteria. These extracts were separated, the active fractions purified, and the active principles isolated and characterized.

Chromatographic separation of the crude ether extract of *G. viscosissimum* led to the isolation and characterization of salicylic acid (**68**), methyl gallate (**69**), and gallic acid (**71**). Similar chromatographic separation of the crude ether and ethyl acetate extracts of *E. umbellatum* resulted in the separation and characterization of fatty acids, methyl gallate, gallic acid, β-sitosterol (**56**), β-sitosteryl-β-D-glucopyranoside (**72**), 3-galloylquinic acid (**74**), and the flavonoids: **77**, **79**, **81**, **83**, **85**, and **87**. Salicylic acid, methyl gallate, gallic acid, and β-sitosterol were identified by comparison with authentic samples. The structure elucidation of β-sitosteryl-β-D-glucopyranoside, 3-galloylquinic acid, and the flavonoids is discussed below.

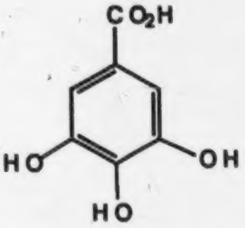
The antibacterial activities of the extracts of *G. viscosissimum* and *E. umbellatum* have been attributed to the presence of methyl gallate. Pure natural methyl gallate demonstrated antibacterial activities against almost all bacteria shown in table 24.



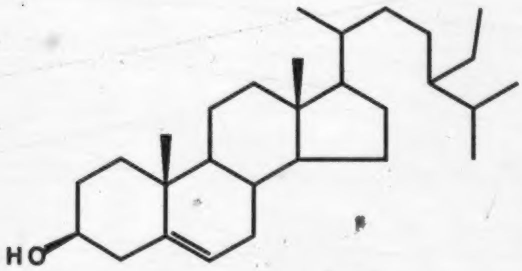
68



69



71



56

Table 24. Antibacterial screening of crude extracts of *G. viscosissimum* and *E. umbellatum* and of 69 and 81.

Organism	*G. viscosissimum* extracts				*E. umbellatum* extracts					
	SkB	Ether	EtOAc (bases)	EtOAc	SkB	Ether	EtOAc (bases)	EtOAc	69	81
Enterobacter cloacae	-	20*	-	-	-	12	-	-	20	-
Escherichia coli	-	-	-	-	-	-	8	-	16	-
Klebsiella pneumoniae	-	10	8	-	-	8	16	8	12	-
Proteus vulgaris	-	10	10	-	-	-	-	8	10	-
Pseudomonas aeruginosa	-	-	-	-	-	-	10	-	10	-
Salmonella typhimurium	-	8	-	-	-	-	-	10	10	-
Serratia marcescens	-	10	-	-	-	-	-	8	10	-
Staphylococcus aureus	8	20	8	10	-	-	-	11	20	
Staphylococcus epidermidis	-	12	8	12	-	-	-	12	14	-
Staphylococcus pyogenes	8	12	10	-	-	-	10	10	-	-
Candida albicans	-	-	-	-	-	-	-	-	Δ	-

* Zone diameter in mm using a 6 mm paper disc.

- No activity.

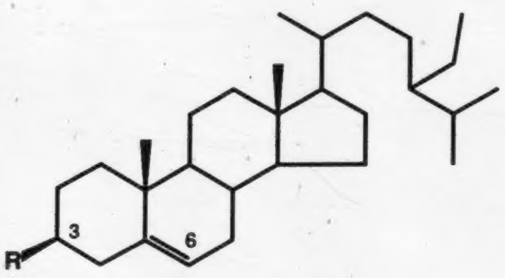
Δ, not tested.

β-Sitosteryl-β-D-glucopyranoside (72).

The crude ether extract of *E. umbellatum* was separated by flash column chromatography (chloroform / ethyl acetate / acetic acid mixtures) and gave methyl gallate and compound **72**. Compound **72** was characterized as its tetraacetyl derivative **73**. Analyses of the spectral properties of compound **73** enabled us to identify it as β-sitosteryl-β-D-tetraacetylglucopyranoside.

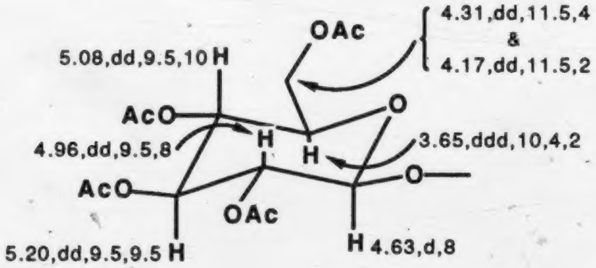
The hreims of each of **72** and **73** displays a fragment ion at m/z 414 ($C_{29}H_{50}O$), suggestive of the molecular ion of β-sitosterol (**56**). Other fragment ions such as m/z 396 ($C_{29}H_{48}$, $M^+ - H_2O$), 381 ($C_{28}H_{45}$, $M^+ - CH_3, -H_2O$), 255 ($C_{28}H_{45}$, M^+ - side chain), and 119 (C_9H_{11}, retro Diels-Alder ring B, $-H_2O$)[47], observed in the hreims of β-sitosterol are also present in the hreims of **72** and **73**. However, both **72** and **73** differ from β-sitosterol in the ^1H nmr spectra. The ^1H nmr spectrum of each of **72** and **73** displays all the ^1H nmr absorption signals of β-sitosterol, as well as absorption signals in the absorption region of carbinyl hydrogens. The signals at δ5.36 (1H, brs, olefinic H-6) and 3.54 (1H, m, carbinyl H-3) in the ^1H nmr spectrum of β-sitosterol resonate at δ5.34 and 3.48, respectively in the ^1H nmr spectrum of **73**. In the ^1H nmr spectrum of **72**, H-6 resonates at δ5.35 and H-3 absorbs at δ3.1-3.6. These ^1H nmr spectral data as well as hreims allow us to conclude that compound **73** is an ether derivative of β-sitosterol, structure **73**.

The R group in **73** was shown to be a β-D-tetra-O-acetylglucopyranoside in the following way. In the ^1H nmr spectrum of tetraacetate **73**, the signal at δ4.63 (1H, d, J = 8 Hz) is assigned to the anomeric hydrogen of β-D-glucose. The anomeric hydrogen in β-D-glucopyranosides absorbs in the same region[56]. The remaining low field hydrogens in the ^1H nmr spectrum of **73** are observed at δ5.20 (1H, dd, J = 9.5,9.5 Hz), 5.08 (1H, dd, J = 10,9.5 Hz), 4.96 (1H, dd, J = 9.5,8 Hz), 4.31 (1H, dd, J = 11.5, 4 Hz), 4.17 (1H, dd, J = 11.5,2 Hz), and 3.65 (1H, ddd, J = 10,4,2 Hz). These signals are of the same spin system and are assigned to hydrogens *geminal* to four acetoxyl groups



73

(δ2.07, 2.04, 2.02, and 2.00, each s, 3H). These hydrogens are assigned as shown in partial structure A. In the ^{13}C nmr spectrum of **73** the signal at δ99.8 is assigned to the anomeric carbon, since in β-D-glucopyranosides the anomeric carbon absorbs in this region[57,58]. The remaining 42 carbons observed in the ^{13}C nmr spectrum of **73** are accounted for by the assigned structure (see Experimental). β-Sitosteryl-β-D-glucopyranoside has been isolated previously in these laboratories from the fungus, *Alternaria brassicae*[59]. The hreims and ^{1}H nmr spectra of the samples of β-sitosteryl-β-D-glucopyranoside isolated from *E. umbellatum* is identical with that previously isolated[59].


OAc
4.31,dd,11.5,4
&
4.17,dd,11.5,2
5.08,dd,9.5,10 H
O
AcO
4.96,dd,9.5,8
H
H
3.65,ddd,10,4,2
AcO
O
OAc
5.20,dd,9.5,9.5 H
H 4.63,d,8

(A)

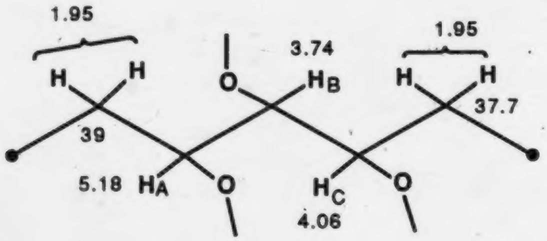
3-Galloylquinic acid (74).

Compound **74** was isolated from the crude ethyl acetate extract of *E. umbellatum* by gel filtration chromatography on Sephadex LH20, using methanol as eluent. Compound **74** is eluted in the earlier fractions from the Sephadex column and on tlc has lower Rf than the flavonoids. The bluish purple colour of **74**, the lack of solubility in almost all organic solvents, as well as the inability to ionize in hreims, presumably due to low volatility indicate that **74** is isolated as a salt and / or a complex of iron. Compound **74** readily dissolves in water. A brown precipitate is formed upon acidification to pH of about 1 and slowly concentrating the aqueous solution at room temperature over several days. The precipitate is separated by filtration through Whatman filter paper. Concentration of the filtrate under reduced pressure affords **74** as an off white solid, which is optically active ($[\alpha]_D = -37^o$) with a mp 235°C (dec.).

The ftir spectrum of compound **74** displays strong hydroxyl(s) (3680-2400 cm^{-1}), carbonyl (1695 cm^{-1}), and carbon-carbon double bond(s) (1595 cm^{-1}). The broad hydroxyl(s) signal is attributed to the presence of a carboxylic acid and / or hydrogen bonded phenolic groups. The signal at δ180.1 in the ^{13}C nmr spectrum of **74** supports the presence of a carboxylic acid and the presence of phenolic groups is confirmed by the blue complex formed between **74** and $FeCl_3$.

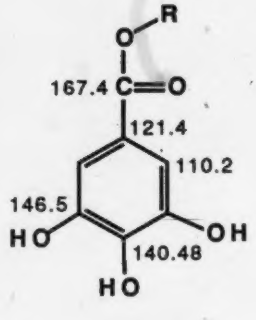
In the ^1H nmr spectrum of **74** the signal at δ 6.99 (s, 2H) is assigned to aromatic hydrogens and these hydrogens indicate the presence of a symmetrically substituted benzene ring. The ^1H nmr spectrum of **74** also displays signals at δ5.18, 4.06, and 3.74, (each 1H) assigned to carbinyl hydrogens, as well as a multiplet signal centered at δ1.95 (4H). Decoupling experiments in the ^1H nmr with **74** indicate that H-A (δ5.18) and H-B (δ3.74) are *vicinal* and *axially* coupled, H-C (δ4.06) is *cis* and *vicinal* to H-B, and that both H-A and H-C are coupled to the hydrogen(s) of the multiplet centered at δ1.95. Upon irradiation of the signal at δ5.18 (brddd, J = 10,10,4.5 Hz), the signal at δ3.74 (brdd, J = 10,3 Hz) collapses to a broad singlet ($W_{1/2}$ = 7 Hz) and the multiplet signal at δ1.95 is perturbed. When the signal at δ3.74 is irradiated, the signal at δ5.18 collapses to a double doublet (J = 10, 4.5 Hz), while H-C at δ4.06 (brd, J = 3 Hz) collapses to a broad singlet ($W_{1/2}$ = 7 Hz). As well, irradiating H-C (δ4.05) perturbs the multiplet signal at δ1.95, while H-B at δ3.74 (brdd, J = 10,3 Hz) collapses to a doublet (J = 10 Hz).

The signals at δ37.7 (t) and 39.0 (t) in the ^{13}C nmr spectrum of **74**, indicate that the four hydrogen multiplet centered at δ1.95 in the ^{1}H nmr spectrum of **74** is due to two methylene groups. These ^{1}H and ^{13}C nmr spectral data allow derivation of partial structure A.

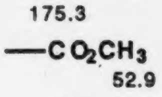


(A)

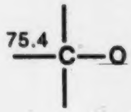
Compound **74** was treated with acidic methanol to afford a methyl ester **75** $C_{15}H_{18}O_{10}$ (358.0870, as shown by cims and hreims). The ftir spectrum of **75** displays hydroxyl(s) (3600-3100 cm^{-1}), methyl ester carbonyl (1727 cm^{-1}), keto or conjugated ester carbonyl (1705 cm^{-1}), and carbon-carbon double (1608 cm^{-1}) bond(s). The signal at δ175.3 in the ^{13}C nmr spectrum of **75** is attributed to the methyl ester carbonyl. The signal at δ167.4 (s) in the ^{13}C nmr spectrum of **75** indicates that the carbonyl signal (1705 cm^{-1}) in the ftir spectrum of **75** is due to an α, β-unsaturated benzoyl group, partial structure B. The absorption at λ_{max} 278 nm in the uv spectrum of **74** supports the presence of partial structure B and this is confirmed by the hreims of **75**. The base peak at m/z 153 ($C_7H_5O_4$) arises from cleavage of the C-O bond of the benzoyl group with concurrent abstraction of a hydrogen atom.


R
O
167.4 C=O
121.4
110.2
146.5
HO
OH
140.48
HO

(B)

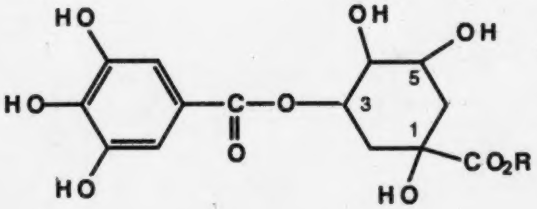

175.3
—CO₂CH₃
52.9

(C)



(D)

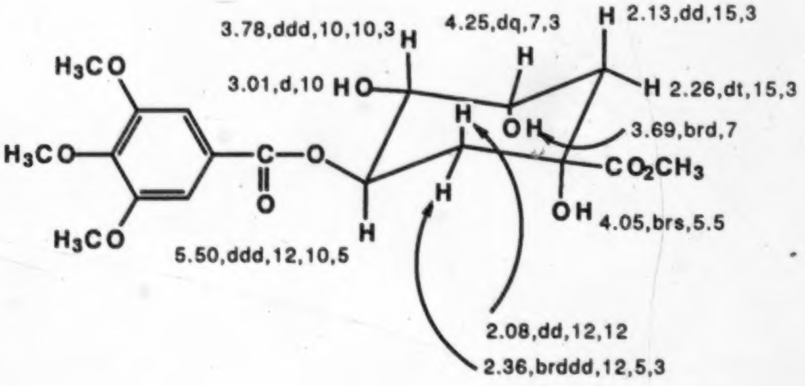
The methyl ester, partial structure C, together with partial structure A and B account for eleven of the twelve signals in the ^{13}C nmr spectrum of **75**. The unassigned signal at δ 75.4 (s) is due to a carbon bearing at least one oxygen atom as shown in partial structure D. Partial structure B and the methyl ester carbonyl account for six of the seven sites of unsaturation indicated by the molecular formula of **75**, thus partial structure A must be cyclic. Partial structures A, B, C, and D allow derivation of structure **75**.


HO
HO
HO
C
O
O
OH
OH
5
3
1
CO₂R
HO

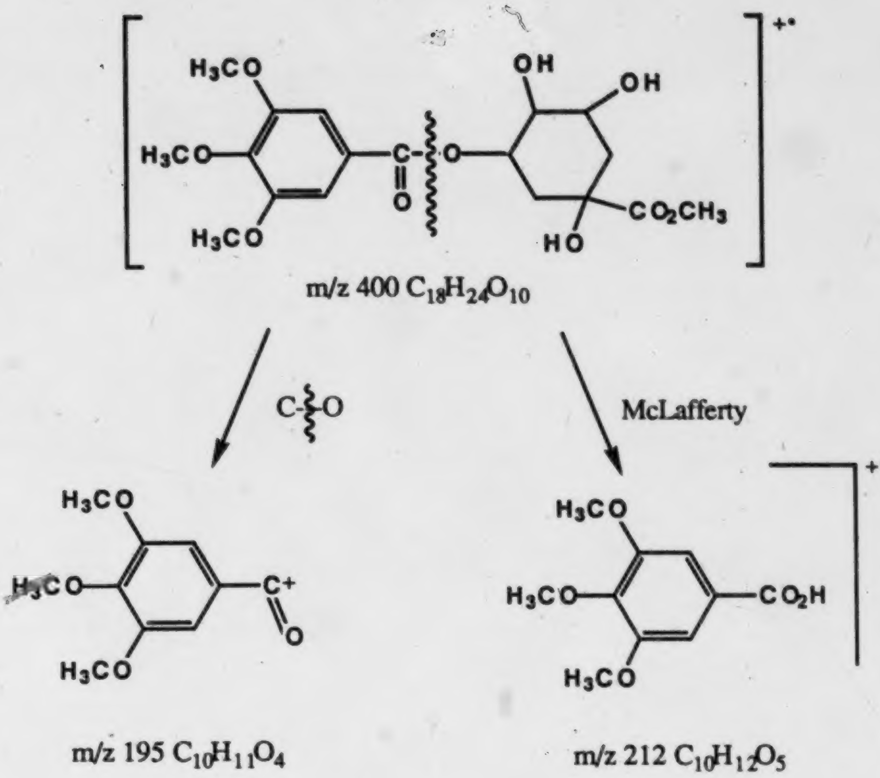
74 R = H
75 R = CH_3

Compound **74** was dissolved in acidic ethanol and treated with diazomethane. A tetramethoxyl derivative **76**, $C_{18}H_{24}O_{10}$ (400.1369, as shown by cims and hreims) was isolated from the reaction mixture. The ftir spectrum of **76** displays hydroxyl(s) (3450 cm^{-1}), methyl ester carbonyl (1730 cm^{-1}), and benzoyl carbonyl (1707 cm^{-1}) groups. The ^1H nmr spectrum of **76** indicates the presence of four methoxyl (δ3.88, 9H, s and 3.80, 3H, s) groups and three D_2O exchangeable hydroxyl hydrogens at δ4.05 (1H, brs, $W_{1/2}$ = 5.5 Hz), 3.69 (1H, brd, J = 7 Hz), and 3.01 (1H, brd, J = 9 Hz). As well, the signals for the cyclohexane ring in the ^1H nmr spectrum of **76** are well resolved compared with those in the ^1H nmr spectra of **74** and **75**. The complete assignment of the hydrogens of the cyclohexane ring is shown in structure **76**. Support for structure **76** arises from its hreims. The peak at m/z 212 ($C_{10}H_{12}O_5$), arising from a McLafferty fragmentation, and the base peak at m/z 195 ($C_{10}H_{11}O_4$), arising from cleavage of the benzoyl C-O bond (scheme 18) are diagnostic.

The spectral data for the parent compound **74** (optical rotation, ^1H and ^{13}C nmr) are identical with those reported for 3-O-galloylquinic acid[60,61].


3.78,ddd,10,10,3
H
4.25,dq,7,3
H
H 2.13,dd,15,3
H₃CO
3.01,d,10 HO
H 2.26,dt,15,3
H
OH
3.69,brd,7
H₃CO
C
O
CO₂CH₃
O
H
OH 4.05,brs,5.5
H₃CO
H
5.50,ddd,12,10,5
2.08,dd,12,12
2.36,brddd,12,5,3

76


m/z 400 $C_{18}H_{24}O_{10}$
C-O
McLafferty
m/z 195 $C_{10}H_{11}O_4$
m/z 212 $C_{10}H_{12}O_5$

Scheme 18. Fragmentation in the hreims of compound **76**

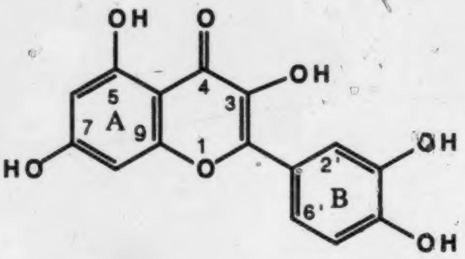
The flavonols 77 and 78.

Compounds **77** and **78** were isolated from the crude basic extract of *E. umbellatum*. The crude basic extract was redissolved in ethyl acetate and the bases were re-extracted from the ethyl acetate solution with 5% HCl (scheme 28, Experimental). The residue ethyl acetate solution was concentrated in *vacuo* to afford an acid / neutral fraction. The acid / neutral fraction afforded a major component **A**, when separated by flash column chromatography.

The ftir spectrum of component **A** displays hydroxyl(s) (strong, broad, *ca* 3500-3100 cm^{-1}), carbonyl(s) (medium, *ca* 1700 cm^{-1}), and aromatic carbon-carbon double bonds (1660, 1603, 1500 cm^{-1}). Component **A** forms an intense blue complex with $FeCl_3$ indicating that the hydroxyl groups are phenolic. Analyses by hreims and ^1H nmr, indicates that component **A** is a mixture of three phenolic compounds in *ca* 2:2:1 ratio (as shown by ^1H nmr).

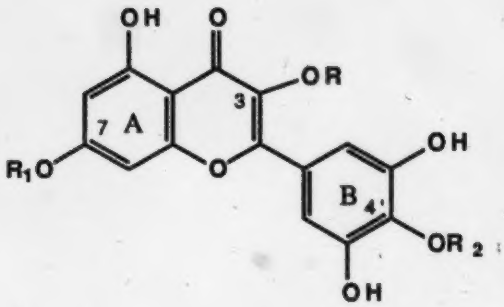
Component one in the mixture **A** is methyl gallate (**69**): hreims: m/z 184 ($C_8H_8O_5$); ^1H nmr: δ7.10 (2H, s) and 3.92 (3H, s). These hreims and ^1H nmr spectral data are identical with those reported for methyl gallate (see Section D3.1, Experimental).

The second component in the mixture **A** is assigned to the known flavonol, quercetin (**77**): hreims and cims: m/z 302 ($C_{15}H_{10}O_7$); ^1H nmr: δ12.18 (1H, brs), 7.84 (1H, d, J = 2.2 Hz), 7.70 (1H, dd, J = 8.5,2.2 Hz), 6.98 (1H, d, J = 8.5 Hz), 6.52 (1H, d, J = 2.2 Hz), and 6.27 (1H, d, J = 2.2 Hz)[62].



77

The third component in the mixture **A** is monomethylated myricetin (**79**): hreims and cims: m/z 332 ($C_{16}H_{12}O_8$); ^1H nmr: δ12.13 (1H, brs), 7.43 (2H, s), 6.68 (1H, d, J = 2 Hz), 6.32 (1H, d, J = 2 Hz), and 4.06 (3H, s). The signal at δ7.43 indicates that ring B in **79** is symmetrically substituted and this together with the signal at δ12.12, which is assigned to the hydroxyl hydrogen at C-5, allow three possible positions (3, 4' or 7) for methyl group substitution. 3-Methylmyricetin, 4'-methylmyricetin, and 7-methylmyricetin have been reported previously[63-67a].



79 R or R_1 or $R_2 = CH_3$
81 R = rha, R_1 or $R_2 = CH_3$
83 R = rha, $R_1 = R_2 = H$

The mixture **A** was acetylated (pyr, Ac_2O) and the acetates were separated by flash column chromatography and preparative tlc. The ^1H nmr of the pentaacetylated methylmyricetin (**80**) differs from that reported for the pentaacetylated 4'-methylmyricetin[63a], thus the methoxyl group in **79** is not attached to C-4'. At this stage the data available does not allow us to distinguish unambiguously between methylation at the 3 or 7 position.

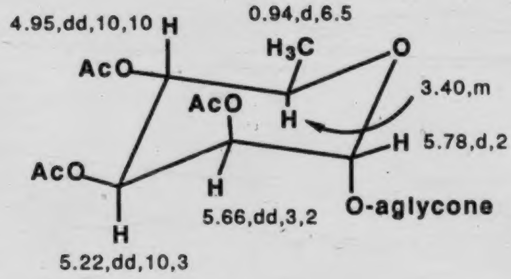
The flavonol glycosides 81, 83, 85, and 87.

The ethyl acetate extract of *E. umbellatum* was separated by gel filtration chromatography on Sephadex LH20. Elution with methanol afforded polar metabolites which make up more than 70% of total crude ethyl acetate extract. The characteristic green to blue complexes formed by these metabolites with $FeCl_3$ indicate their phenolic nature. The purification and identification of these metabolites was complicated by their lack of solubility in most organic solvents. To simplify purification, impure fractions obtained by column chromatography were acetylated and the component acetates were separated and characterized as the acetyl derivatives. Compound **81** was identified as the free alcohol as well as its acetyl derivative **82**, whereas compounds **83**, **85**, and **87** were separated and characterized as the acetyl derivatives, **84**, **86**, and **88**, respectively.

The ftir spectrum of compound **81** displays strong and broad intensity bands attributed to hydroxyl(s) (3431-3217 cm^{-1}) and medium intensity bands assigned to carbonyl or carbon-carbon double bond(s) (1661 and 1559 cm^{-1}). The base peak at m/z 332 ($C_{16}H_{12}O_8$) in the hreims of **81** is similar to the parent ion in the hreims of methylmyricetin, compound **79**. However, compound **81** is optically active ($[\alpha]_D$ = -154°) and this suggests that **81** is composed of an optically active group attached to methylmyricetin (**79**) or its isomer.

The ^1H nmr spectrum of compound **81** displays absorption signals at δ12.8 (1H, s), 6.87 (2H, s), 6.65 (1H, d, J = 2 Hz), 6.40 (1H, d, J = 1 Hz), and 3.94 (3H, s), similar to those observed in the ^1H nmr spectrum of **79**. In addition, the ^1H nmr spectrum of **81** has signals at δ5.21 (1H, brs, $W_{1/2}$ = 3 Hz), 4.12 (1H, brs, $W_{1/2}$ = 6.5 Hz), 3.65 (1H, brdd, J = 9, 3 Hz), 3.47 (1H, m), 3.25 (1H, dd, J = 9, 9 Hz), and 0.99 (3H, d, J = 6 Hz). These signals are assigned to the hydrogens of the monosaccharide rhamnose. The signal at δ5.21 is assigned to the anomeric hydrogen and its coupling pattern ($W_{1/2}$ = 3 Hz) indicates that it is the α-L anomer of rhamnose[68]. This assignment is confirmed by the ^1H nmr spectrum of the hexaacetyl derivative of **81**, compound **82**. The signals due to the rhamnosyl group are well resolved and are assigned as shown in partial structure A.

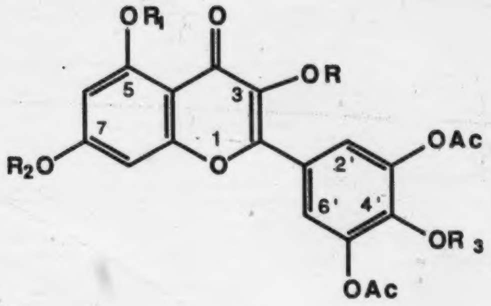
In the ^1H nmr spectra of **81** and **82**, the signals at δ6.87 (**81**) and 7.70 (**82**) (each 2H, s) are assigned to H-2' and H-6' and this indicates that ring B is symmetrically substituted. This allows us to conclude that in compound **81**, the rhamnose is attached to the flavonol at position 3 or 7 and the methoxyl group is at position 3, or 4', or 7. The coupling constant ($J = 2$ Hz) for the anomeric hydrogen (H-1") in **82** and the coupling constant ($J \equiv 6$ Hz) for the rhamnosyl methyl group (H-6") in **81** and **82** indicate that the sugar is attached to C-3. The hydrogens, H-1" and H-6" in flavonol 7-O-rhamnosides appear as complex multiplets due to free rotation about the O-C-1" bond and these hydrogens are sharper in flavonol 3-O-rhamnosides due to more restricted rotation[68]. The ^{13}C nmr spectrum of **81** provides support for this assignment. The signal at δ 134.5 assigned to C-3 in the ^{1}H nmr spectrum of **81** is identical with that reported for myricetin 3-O-rhamnoside, compound **83** (see table 25)[67b]. Since the sugar is attached to C-3, the methoxyl group is attached to C-4' or C-7. The ^{13}C nmr spectral data for compound **81** when compared to the ^{13}C nmr data reported for myricetin 3-O-rhamnoside (**83**) (table 25) appear to favour methoxyl substitution at C-7. The chemical shifts for C-1' to C-6' in **81** and **83** are identical and this indicates that compounds **81** and **83** have an identical ring B. However, the shieldings (Δ) observed for ring A carbons C-5 to C-8 in **81** (with the methoxyl group attached to C-7) when compared to **83** are small. Such shieldings are of the order +2-3 ppm[69]. 4'-Methylmyricetin 3-O-rhamnoside and 7-methylmyricetin 3-O-rhamnoside are known compounds. To our knowledge no ^{1}H or ^{13}C nmr spectral data have been reported[70-72], thus unambiguous distinction between methylation at position 4' or 7 still remains unresolved.



(A)

Table 25 ^{13}C nmr spectra of **81**and **83** (DMSO-d_6).

C	Chemical shift in δ 83 (25.15 MHz)	 81 (100.6 MHz)	Δ
4	177.7	177.7	0
7	164.1	165.0	+0.9
5	161.3	160.9	-0.4
2	157.4	157.7	+0.3
9	156.4	156.2	-0.2
3'&5'	145.8	145.6	-0.2
4'	136.5	136.4	-0.1
3	134.5	134.4	-0.1
1'	119.9	119.0	-0.9
2'&6'	108.2	108.0	-0.2
10	104.2	104.9	+0.7
1"	102.0	101.8	-0.2
6	98.7	97.6	-1.1
8	93.5	92.0	-1.5
4"	71.5	71.2	
2"	70.5	70.3	
3"	70.6	70.3	
5"	70.1	69.8	
6"	17.8	17.2	
O$\underline{C}H_3$		55.8	



82 R = rha, R_1 = H, R_2or R_3= CH_3 or Ac
84 R = rha, $R_1 = R_2 = R_3$ = Ac
86 R = β-D-pyranoside, $R_1 = R_2 = R_3$ = Ac

An octaacetyl derivative, compound **84**, is an optically active compound ($[\alpha]_D$= -167°). The ftir spectrum of **84** displays aromatic acetoxyl carbonyl(s) (1780 cm^{-1}), aliphatic acetoxyl carbonyl(s) (1750 cm^{-1}), and carbon-carbon double bond(s) (1647 and 1628 cm^{-1}). The presence of aromatic and aliphatic acetoxyl groups is confirmed in the ^{1}H nmr spectrum of **84** by the signals at δ2.43 (3H, s), 2.32 (6H, s), and 2.30 (3H, s) (aromatic acetoxyl methyls) and δ2.00 (3H, s), 2.14 (3H, s), and 1.98 (3H, s) (aliphatic acetoxy methyls). Except for the absence of a methoxyl group, the remaining signals in the ^{1}H nmr spectrum of **84** are similar to those observed in the ^{1}H nmr spectrum of compound **82** (see table 26). These ^{1}H nmr spectral data indicate that **84** is 2",3',3",4',4",5,5',7-octaacetylmyricetin 3-O-rhamnoside. The ^{13}C nmr spectrum of **84** shows the 34 signals of the carbon skeleton of structure **84**. The spectral data (optical rotation and ^{1}H nmr) for compound **84** are identical with those reported for 2",3',3",4',4",5,5',7-octaacetylmyricetin 3-O-rhamnoside **84**[70].

Table 26. ^1H nmr spectra of **82** and **84** ($CDCl_3$, 400 MHz).

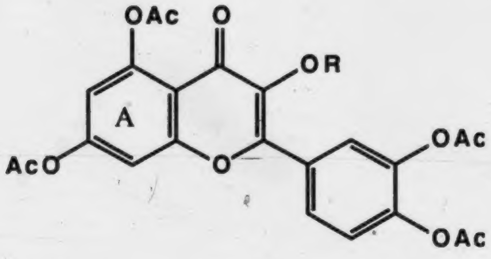
Chemical shift in δ, mult., J in Hz.

H	82	84
5-$\underline{H}$O-	11.37, s	
2'&6'	7.70, s	7.69, s
8	6.44, d, 2	7.30, d, 2
6	6.38, d, 2	6.85, d, 2
1"	5.78, d, 2	5.74, d, 2
2"	5.66, dd, 3,2	5.67, dd, 3,2
3"	5.22, dd, 10,3	5.18, dd, 10,3
4"	4.95, dd, 10,10	4.92, dd, 10,10
5"	3.40, m	3.32, m
$OC\underline{H}_3$	3.87, s	
$C\underline{H}_3CO_2$-		2.43, s
"		2.34, s
2 x $C\underline{H}_3CO_2$-	2.32, s	2.32, s
$C\underline{H}_3CO_2$-	2.30, s	2.30, s
"	2.14, s	2.14, s
"	2.01, s	2.00, s
"	1.98, s	1.99, s
6"	0.94, d, 6	0.93, d, 7

A nonaacetyl derivative, compound **86** is an optically active compound ($[\alpha]_D = -72^o$) and shows similar ftir spectral data to that of octaacetate **84**: aromatic acetoxyl carbonyl(s) (1779 cm^{-1}), aliphatic acetoxyl carbonyl(s) (1752 cm^{-1}), and carbon-carbon double bond(s) (1645 and 1628 cm^{-1}). The signals at δ7.87 (2H, s), 7.33 (1H, d, J = 12 Hz), and 6.85 (1H, d, J = 2 Hz) in the ^{1}H nmr spectrum of **86**, indicate that the flavonol part of **86** is similar to that of **82** and **84**, thus, **86** is a glycoside of myricetin. The presence of four aliphatic acetoxyl groups at δ2.16 (3H, s), 2.14 (3H, s), 2.00 (3H, s), and 1.91 (3H, s) in the ^{1}H nmr spectrum of **86** suggests that the saccharide is a hexose. The anomeric hydrogen (δ5.56, d) and its coupling constant of J = 8 Hz indicates that the aglycone is bonded through a β-linkage[68]. The signal at δ99.5 (d) in the ^{13}C nmr spectrum of **86** is assigned to the anomeric carbon, since the chemical shift of the anomeric carbon in flavonol β-D-glycosides appear in the same region[67c].

A fourth peracetyl glycoside, octaacetate **88** is optically active ($[\alpha]_D = -88^o$) and has ftir spectral data similar to that of compound **84** and **86**: aromatic acetoxyl carbonyl (1777 cm^{-1}), aliphatic acetoxyl carbonyl (1753 cm^{-1}), and carbon-carbon double bond(s) (1764 and 1628 cm^{-1}). The signals at δ7.98 (1H, dd, J = 9,2 Hz), 7.92 (1H, d, J = 2 Hz), 7.33 (1H, d, J = 9 Hz), 7.32 (1H, d, J = 2 Hz), and 6.84 (1H, d, J = 2 Hz) in the ^{1}H nmr spectrum of compound **88** suggest that the aglycone is quercetin (**77**). The signals for the sugar in the ^{1}H and ^{13}C nmr spectra of **88** are similar to those of the sugar in **86** (see Experimental). The signals for the anomeric hydrogen (δ5.48, 1H, d, J = 8 Hz) and the anomeric carbon (δ99.6) in the ^{1}H nmr spectrum of **88** are identical to those observed in the ^{1}H and ^{13}C nmr spectra of **86**: δ5.56 (1H, d, J = 8 Hz) and 99.5, respectively, indicating that the saccharide and glycosidic linkage in **86** and **88** are the same i.e.,β-D-glycosidic linkage. The position of attachment to the flavonol in **88** is probably at C-3. This is supported by ^{1}H nmr spectral data of **88** and pentaacetylquercetin **78** (table 27). The signals for the ring A hydrogens in **78** and **88** are identical. This would not be the case if the glycoside was attached to ring A.

Furthermore, the shieldings, (Δ) for H-2' and H-6' between **78** and **88** are nearly the same, thus disfavoring sugar attachment to ring B. Therefore, **86** is 2",3',3",4',4",5,5',6",7-nonaacetylmyricetin 3-O-β-D-glycoside and **88** is 2",3',3",4',4",5,6",7-nonaacetylquercetin 3-O-β-D-glycoside. The identity of the saccharide in both **86** and **88** is still unresolved.



88 R = β-D-pyranoside

Table 27. Comparison of some ^{1}H nmr spectral data of **78** and **88** ($CDCl_3$, 200 MHz).

Chemical shift in δ mult. J in Hz.

H	78	88	Δ
2'	7.69, d, 2.5	7.92, d, 2	+0.23
6'	7.72, dd, 8,2.5	7.98, dd, 9,2	+0.26
5'	7.36, d, 8	7.33, d, 9	-0.03
8	7.34, d, 2.5	7.32, d, 2	-0.02
6	6.89, d, 2.5	6.84, d, 2	-0.05

V. Experimental.

A. General.

High resolution electron impact mass spectra (hreims) were recorded on an AEI MS-50 mass spectrometer coupled to a DS-50 computer. Data is reported as m/z (formula, relative intensity, fragment). Unless diagnostically significant, peaks with intensities less than 20% of the base peak are omitted. Chemical ionization mass spectra (cims) were recorded on an AEI MS-12 mass spectrometer with ammonia as the reagent gas. Gas-chromatography / mass-spectra (gc / ms) were recorded on a Varian vista 600 equipped with a mass spectrometry detector 7070E. Fourier transform infrared (ftir) spectra were recorded (as $CHCl_3$ cast unless otherwise noted) on a Nicolet FT 7199 interferometer. Ultraviolet (uv) spectra were recorded on a Hewlett Packard 8450A diode array or a Unicam sp 1700 spectrophotometer. Optical rotations were recorded on a Perkin Elmer 141 polarimeter. ^{1}H and ^{13}C Nuclear magnetic resonance (nmr) spectra were measured (in $CDCl_3$ unless otherwise noted) on Brucker WH-200, WH-300, WH-360 or WH-400 spectrometers. Chemical shifts are reported in parts per million (δ value from tetramethylsilane (TMS)). Chloroform (unless otherwise noted) was employed as internal standard, ^{1}H: δ7.26, ^{13}C: 77.00 relative to TMS. Coupling constants, J, are expressed in cycles per second (Hertz, Hz) and the following abbreviations are used: m = multiplet, s = singlet, d = doublet, t = triplet, q = quartet, br = broad. Melting points are uncorrected and were measured on a Zeitz-Wetzlar or a Thomas Model 40 melting point apparatus.

Reagent grade solvents were distilled prior to use. Skellysolve B refers to Skelly oil company light petroleum, bp 62-70°C. Analytical grade diethyl ether (ACS 288) was used without further purification. Pyridine was distilled from NaOH and stored over NaOH. Acetic anhydride was dried over P_2O_5 and distilled over sodium acetate.

Woelm (activity III/20 mm) and Brockman (activity I and II) neutral alumina was used for column chromatography. Fractions were collected with an ISCO model 1200 or model 820 fraction collector. E. Merck silica gel 60 (230-400 mesh) was used for flash column chromatography[73]. Pharmacia Sephadex LH20 and G10 was used for gel filtration chromatography. Analytical thin-layer chromatography (tlc) was carried out on silica gel precoated tlc plates (E. Merck DC-Alufolien, Kieselgel 60 F-254 0.2 mm thickness) and on alumina precoated tlc plates (E. Merck DC-Alufolien, Aluminumoxide 60 F254 neutral type E 0.2 mm thickness). The chromatograms were examined under ultraviolet light (254 or 350 nm). Visualization was completed by spraying with Dragendorrf's reagent (solution A: 1.7 g of basic $Bi(NO_3)_3.5H_2O$ in 100 mL of water: acetic acid; 4:1; solution B: 40 g of KI in 100 mL of water; spray solution: 20 mL of solution A, 5 mL of solution B, and 70 mL of water) or by dipping in a 3% phosphomolybdic acid solution (3 g of $20MoO_3.2H_3PO_4.48H_2O$, 0.5 g of $H_4Ce(SO_4)_4$ in 100 mL of 3% (v/v) sulphuric acid).

B. *Lycopodium obscurum.*

B1. Plant collection and extraction.

L. obscurum was collected in eastern Canada during early September 1986 from York County, New Brunswick by Dr. H. Hinds. The sample from western Canada was collected from Alberta in two batches by our group. The first batch was collected during late October 1986 from Fox Creek at Pine Tree campgrounds and the second batch was collected during early September 1987 from Fox Creek at Pine Tree campgrounds and

125 km south of Grand Prairie on Forestry road 734. Specimens* are deposited in the herbarium, Department of Botany, University of Alberta.

The whole plant of *L. obscurum* was air dried for several weeks (7 weeks to 1 year) before pulverization and extraction. In a second extraction, whole plant collected in western Canada was air dried for 3 weeks, ground, and extracted immediately. The less stable Lycopodium alkaloids were isolated only from the second crude extract.

Ground plant was extracted as described below by Soxhlet extraction with methanol[74] or by percolation with 2% tartaric acid[39]. The latter extraction procedure afforded cleaner and better yields of crude alkaloid mixture than the former (0.12% and 0.10% by percolation with tartaric acid and by Soxhlet extraction, respectively).

Dry ground plant (340 g) of *L. obscurum* from eastern Canada was extracted by percolation with tartaric acid to afford 420 mg of crude bases. This crude base mixture was analysed by gc / ms.

B1.1. Soxhlet extraction .

Pulverized plant (*ca* 1.9 kg) was extracted with methanol for 48 hrs according to scheme 19. The methanol extract was concentrated under reduced pressure at 40°C. The concentrate was transferred to a large beaker, diluted onefold with water, and the remainder of the methanol removed by evaporation on a steam bath. The mixture was digested (diluted with 10% (v/v) HCl and heated to boiling on a steam bath) and then cooled to room temperature. The solution was filtered through celite and the celite was washed thoroughly with 5% (v/v) HCl. The aqueous extract was extracted successively with Skellysolve B and ether to remove neutral and acidic compounds. The aqueous residue was basified to pH of approximately 11 (aq NH_3) and the bases were extracted successively with ether, dichloromethane, and ethyl acetate. The total crude bases (*ca* 2.7 g) were redissolved in 2% (w/v) tartaric acid and re-extracted according to scheme 20. The ether and dichloromethane layers were washed with water, dried (anhydrous $MgSO_4$ or Na_2SO_4), and evaporated to dryness (in *vacuo*) to afford crude bases (*ca* 1.9 g) in 0.1% yield.

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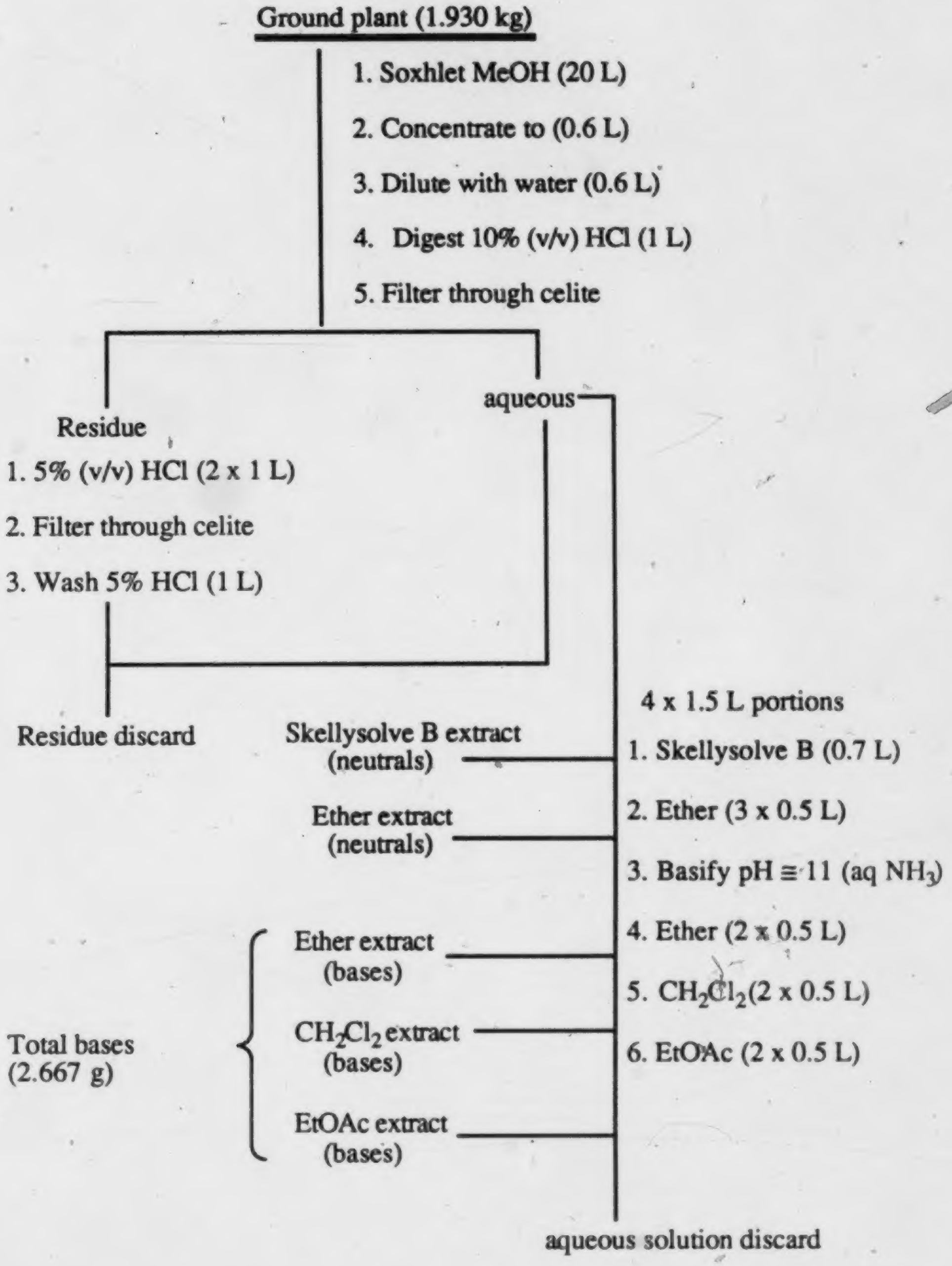
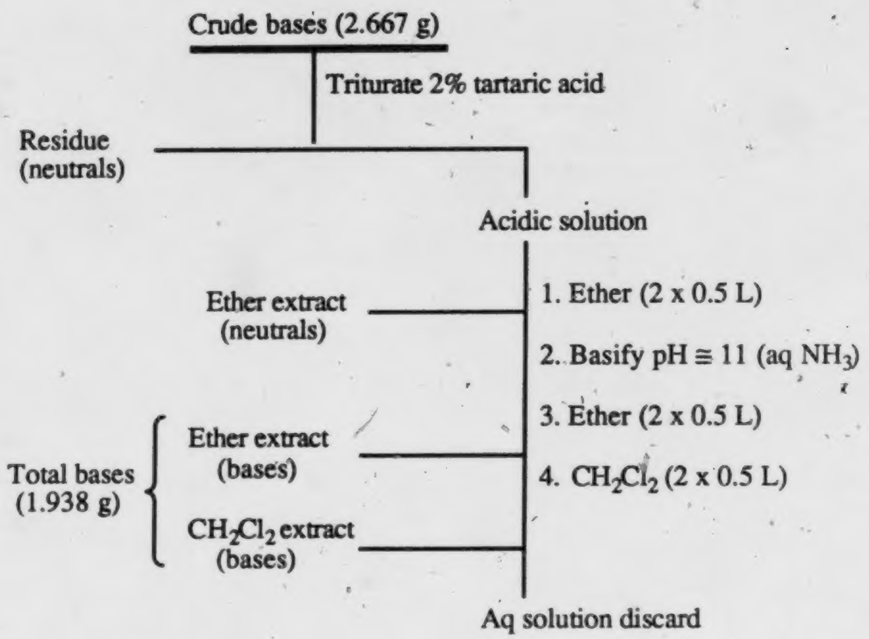
* Accession numbers : 91187 (York County), 91186 (Fox Creek,1986), 92370 (Fox Creek, 1987), and 92371 (Grand Prairie, 1987).

B1.2. Extraction with tartaric acid.

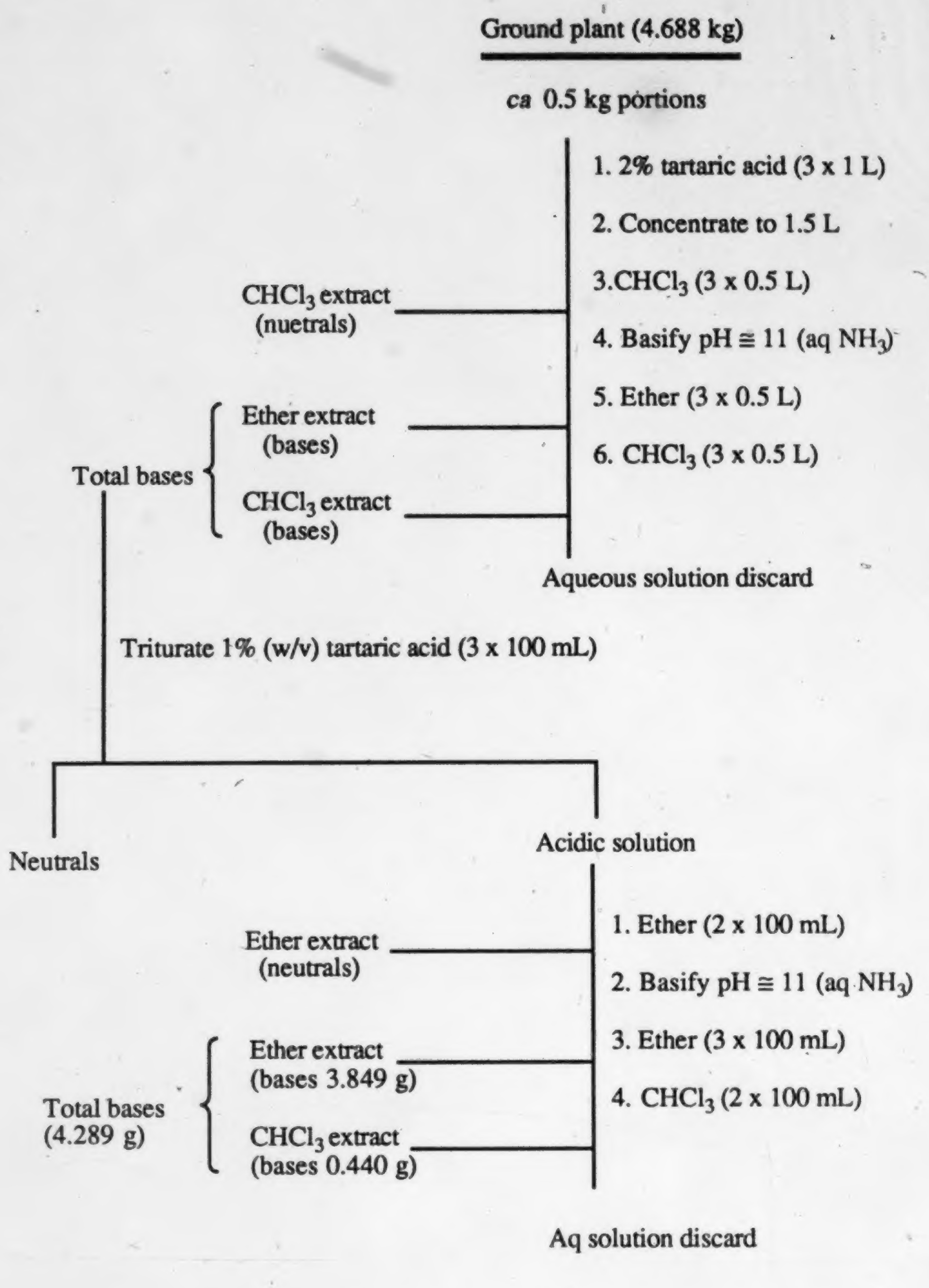
L. obscurum was extracted with 2% tartaric acid according to scheme 21. Dry ground plant was suspended in 2% aqueous tartaric acid in a 4 L Erlenmeyer flask fitted with a stopcock at the bottom. The mixture was constantly agitated with a motor driven mechanical stirrer at room temperature for 24 hrs. The suspension was allowed to settle, the solution drained, and filtered. The extraction with tartaric acid was repeated 3 times. The extracts were combined and concentrated in *vacuo* at 50°C during which time a white tartarate salt precipitated. The salt was removed and the tartaric acid solution (6 L) was divided into 4 equal positions. Each portion was extracted with chloroform (X 3) to remove neutral and acidic compounds and basified to pH 11 (aq. NH_3). The basic solution was extracted successively with ether and chloroform. Each of the ether and chloroform layers was washed with water, dried ($MgSO_4$), and concentrated to yield the crude bases (4.2887 g) in 0.1% yield.


Ground plant (1.930 kg)
1. Soxhlet MeOH (20 L)
2. Concentrate to (0.6 L)
3. Dilute with water (0.6 L)
4. Digest 10% (v/v) HCl (1 L)
5. Filter through celite
Residue
1. 5% (v/v) HCl (2 x 1 L)
2. Filter through celite
3. Wash 5% HCl (1 L)
aqueous
Residue discard
4 x 1.5 L portions
Skellysolve B extract (neutrals)
1. Skellysolve B (0.7 L)
Ether extract (neutrals)
2. Ether (3 x 0.5 L)
3. Basify pH ≅ 11 (aq NH3)
Ether extract (bases)
4. Ether (2 x 0.5 L)
CH2Cl2 extract (bases)
5. CH2Cl2 (2 x 0.5 L)
Total bases (2.667 g)
EtOAc extract (bases)
6. EtOAc (2 x 0.5 L)
aqueous solution discard

Scheme 19. Extraction of *L. obscurum* with methanol in a Soxhlet apparatus.


Crude bases (2.667 g)
Triturate 2% tartaric acid
Residue (neutrals)
Acidic solution
1. Ether (2 x 0.5 L)
2. Basify pH ≅ 11 (aq NH_3)
3. Ether (2 x 0.5 L)
4. CH_2Cl_2 (2 x 0.5 L)
Ether extract (neutrals)
Total bases (1.938 g)
Ether extract (bases)
CH_2Cl_2 extract (bases)
Aq solution discard

Scheme 20. Re-extraction of the crude bases of *L. obscurum* with 2% (w/v) tartaric acid.


Ground plant (4.688 kg)
ca 0.5 kg portions
1. 2% tartaric acid (3 x 1 L)
2. Concentrate to 1.5 L
3.$CHCl_3$ (3 x 0.5 L)
4. Basify pH ≅ 11 (aq NH_3)
5. Ether (3 x 0.5 L)
6. $CHCl_3$ (3 x 0.5 L)
$CHCl_3$ extract (nuetrals)
Ether extract (bases)
Total bases
$CHCl_3$ extract (bases)
Aqueous solution discard
Triturate 1% (w/v) tartaric acid (3 x 100 mL)
Neutrals
Acidic solution
1. Ether (2 x 100 mL)
2. Basify pH ≅ 11 (aq NH_3)
3. Ether (3 x 100 mL)
4. $CHCl_3$ (2 x 100 mL)
Ether extract (neutrals)
Ether extract (bases 3.849 g)
Total bases (4.289 g)
$CHCl_3$ extract (bases 0.440 g)
Aq solution discard

Scheme 21. Extraction of *L. obscurum* by percolation with 2% (w/v) tartaric acid.

B2. Gas-chromatography/mass-spectrometry.

A silica capillary column (30 m x 0.25 mm) DB-5 or DB-1 (J & W) was used for gas chromatographic analysis. The conditions were: carrier gas: He at a pressure of 12 lb / square inch and a flow rate of 1.5 mL / minute; temperature program: 150-250°C at 5° / minute and 250-300°C at 20° / minute. A standard solution containing 0.1 mg in each of ten authentic Lycopodium alkaloids shown in table 2 in methanol (0.1 mL) was used to standardize the conditions. The concentration was 1mg / mL methanol in each sample of *L. obscurum* from eastern and western Canada. The standard mixture and each sample of *L. obscurum* was introduced in the inlet chamber at 2 μL injections. The gc / ms results with the authentic samples of Lycopodium alkaloids are shown in table 2.

B3. Isolation.

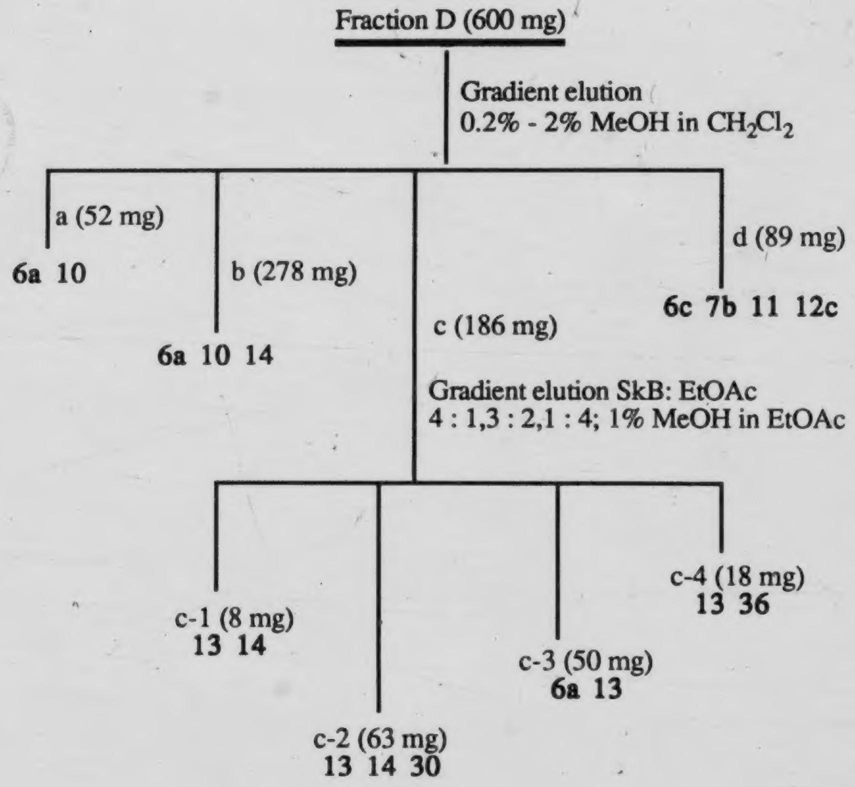
The crude alkaloid mixture (5.0647 g) was partitioned into fractions **A-G** (table 28) by gradient elution chromatography over alumina (Woelm, 400 g, 40 mm id column, methanol / dichloromethane). Eluent volumes of 20 to 25 mL were collected. Each of the fractions **A-G** was purified further by gradient elution over alumina, or by flash column chromatography over silica gel, or by preparative tlc. Preparative tlc was performed on silica gel (unless otherwise noted) and the bases were extracted from silica gel with methanol in dichloromethane presaturated with ammonia. The purity of the chromatography fractions was monitored by tlc using alumina plates.

Table 28. Preliminary separation of the crude base extract of *L. obscurum*.

Solvent(volume,L)	Fraction	Yield (g)	Observation
0.3% MeOH/CH_2Cl_2(0.5)	A	2.242	**6a**
0.5% " "	B	0.776	**6a**
1.0% " (1.0)	C	0.172	**6a,10**
	D	0.755	**6a,6c,7b,10,11, 12c,13,14,30,36**
2.0% " (1.0)	E	0.204	**6b,7c,12c,27,29**
3.0% " (0.5)	F	0.239	**6b,7c,7d,8,27, 29,38,43**
5.0% " (0.5)	G	0.213	**7a,12d,27,29,53**
20.0% " (0.5)		"	"

B3.1. Isolation of lycopodine (6a) and lycodine (10).

Fraction A (2.243 g) contains mainly lycopodine (**6a**) and two unidentified alkaloids of molecular weight 244 and 245 (gc / ms). Lycopodine was identified by comparison with an authentic sample (mp, hreims, ^{1}H and ^{13}C nmr spectra)[3a,30,75]. Fraction B (0.776 g) contains lycopodine. Fraction **C** (0.172 g) contains lycopodine and lycodine (**10**). Fraction **C** was separated by flash column chromatography (acetone:dichloromethane; 1:4, 1:1, and 1% methanol in dichloromethane presaturated with ammonia) to afford lycodine (*ca* 100 mg) and lycopodine (*ca* 60 mg). Lycodine was recrystallized from hexane as colourless plates, mp: 112°C (118°C lit[3a]); tlc: Rf 0.51 (2% methanol in dichloromethane); ftir υ_{max} cm^{-1}: 3280 (weak, NH), 1571; uv ($\underline{c}$ = 0.82, ethanol) λ_{max} nm (log ε): 268 (3.8) 276 (3.7); $[\alpha]_D$ = -8° ($\underline{c}$ = 0.79, methanol); ^{1}H and ^{13}C nmr: see tables 12 and 13; hreims: m/z calcd. for $C_{16}H_{22}N_2$: 242.1785, found: 242.1783 (17, M$^+$), 185 ($C_{12}H_{13}N_2$, 100, M$^+$-C_4H_9), 157 ($C_{10}H_9N_2$, 10, M$^+$-C_4H_9, -C_2H_4).


Fraction D (600 mg)
Gradient elution
0.2% - 2% MeOH in CH2Cl2
a (52 mg)
6a 10
b (278 mg)
6a 10 14
c (186 mg)
d (89 mg)
6c 7b 11 12c
Gradient elution SkB: EtOAc
4 : 1,3 : 2,1 : 4; 1% MeOH in EtOAc
c-1 (8 mg)
13 14
c-2 (63 mg)
13 14 30
c-3 (50 mg)
6a 13
c-4 (18 mg)
13 36

Scheme 22. Isolation of obscurinine (**13**), iso-obscurinine (**14**), acetyllobscurininol (**30**), hydroxypropyllycodine (**36**), flabellidine (**11**), and flabelliformine (**6c**).

B3.2. Isolation of compounds 6c, 11, 13, 14, 30, and 36.

A portion of fraction **D** (600 mg) was separated by gradient elution chromatography over alumina (100 g) using methanol in dichloromethane (0.2 to 2%) into 4 fractions **a** to **d** as shown in scheme 22. Each of fractions **b**, **c**, and **d** was purified further.

Iso-obscurinine (14) and obscurinine (13).

Iso-obscurinine (4 mg) and obscurinine (85 mg) were obtained from a portion of fraction **b** (169 mg) by flash column chromatography using acetone:dichloromethane; 1:1.

Iso-obscurinine (14).

Iso-obscurinine crystallizes on slow evaporation from Skellysolve B / acetone as off white plates, mp: 106-107°C; tlc: Rf 0.49 (Skellysolve B:ethyl acetate, 4:1); $[\alpha]_D = +107^\circ$ ($\underline{c}$ = 1.1, methanol); ftir υ_{max} cm^{-1}: 2964, 2924, 2853, 2838, 2786 (*Bohlmann bands*) 1734, 1649, 1605; ^1H and ^{13}C nmr: see tables 3 and 4; hreims: m/z calcd. for $C_{17}H_{24}N_2O$: 272.1890; found: 272.1888(100, M^+), 271 ($C_{17}H_{23}NO$, 5, M^+-H), 257 ($C_{16}H_{21}N_2O$, 15, M^+-CH_3), 243 ($C_{16}H_{23}N_2$, 3, M^+-HCO), 189 ($C_{12}H_{15}NO$, 5, M^+-C_5H_9N), 173 ($C_{12}H_{15}N$, 20, M^+-C_5H_9NO), 172 ($C_{12}H_{14}N$, 21, M^+-$C_5H_{10}NO$), 160 ($C_{11}H_{14}N$, 14, M^+-$C_6H_{10}NO$), 159 ($C_{11}H_{13}N$, 14, M^+-$C_6H_{11}NO$), 158 ($C_{11}H_{12}N$, 13, M^+-$C_6H_{12}NO$), 146 ($C_{10}H_{12}N$, 15, M^+-$C_7H_{12}NO$), 98 ($C_6H_{12}N$, 36), 84 ($C_5H_{10}N$, 51), 72 ($C_4H_{10}N$, 66), 71 (C_4H_9N, 64), 70 (C_4H_8N, 30), 58 (C_3H_8N, 88), 57 (C_3H_7N, 30).

Obscurinine (13).

Obscurinine crystallizes on slow evaporation from Skellysolve B / acetone as off white plates, mp: 128-130°C (124-125.2°C, lit[19]); tlc: Rf 0.37 (Skellysolve B:ethyl acetate; 4:1); $[\alpha]_D$ = +242° (c = 0.5, methanol); uv (c = 0.43, ethanol) λ_{max} nm (log ε): 205 (3.9), 233 (4.0); ftir υ_{max} cm^{-1}: 3029, 3006 (CH, olefinic), 2963, 2944, 2936, 2912, 2838, 2809, 2801, 2792, 2765, 2719, 2708 (*Bohlmann bands*), 1733, 1646, 1627, 1603; ^{1}H and ^{13}C nmr: see tables 3 and 4; cims: m/z 273 (100, M^++1); hreims: m/z calcd. for $C_{17}H_{24}N_2O$: 272.1890, found: 272.1886 (100, M^+), 271 ($C_{17}H_{23}NO$, 3, M^+-H), 257 ($C_{16}H_{21}N_2O$, 15, M^+-CH_3), 244 ($C_{16}H_{24}N_2$, 2, M^+-CO), 189 ($C_{12}H_{15}NO$, 10, M^+-C_5H_9N), 172 ($C_{12}H_{14}N$, 10, M^+-$C_5H_{10}NO$), 160 ($C_{11}H_{14}N$, 16, M^+-$C_6H_{10}NO$), 159 ($C_{11}H_{13}N$, 12, M^+-$C_6H_{11}NO$), 158 ($C_{11}H_{12}N$, 12, M^+-$C_6H_{12}NO$), 146 ($C_{10}H_{12}N$, 17, M^+-$C_7H_{12}NO$), 98 ($C_6H_{12}N$,18), 84 ($C_5H_{10}N$, 34), 72 ($C_4H_{10}N$, 61), 71 (C_6H_9N, 62), 70 (C_4H_8N, 22), 58 (C_3H_8N, 39), 57 (C_3H_7N, 18).

Acetyllobscurinol (30).

Acetyllobscurinol (30) (3.5 mg) was obtained as a colourless opaque solid from fraction c-2 (63 mg) (scheme 22) by flash column chromatography (acetone:dichloromethane; 1:4, 1:1, and 1% methanol in dichloromethane presaturated with ammonia) and by preparative tlc (3% methanol in ether under an atmosphere of ammonia), tlc: Rf 0.62 (2% methanol in ethyl acetate) or 0.90 (5% methanol in ether/ammonia vapours, silica gel); $[\alpha]_D$= +110° (c = 0.16, methanol); uv (c = 0.015, ethanol) λ_{max} nm (log ε): 202 (3.2) and 234 (3.3); ftir υ_{max} cm^{-1}: 2956, 2925, 2854, 1735, 1659, 1240; ^{1}H and ^{13}C nmr: see tables 7 and 8; cims: m/z 318 (100, M^++1); hreims: m/z calcd. for $C_{19}H_{27}NO_3$: 317.1992, found: 317.1985 (18, M^+), 274

($C_{17}H_{24}NO_2$, 5, M^+-CH_3,-CO), 258 ($C_{17}H_{24}NO$, 100, M^+-CH3CO_2), 257 ($C_{17}H_{23}NO$, 8, M^+-CH_3CO_2H), 215 ($C_{15}H_{19}O$, 39), 187 ($C_{13}H_{15}O$, 7, M^+-CH_3CO_2H,-C_4H_8N), 84 ($C_5H_{10}N$, 38), 83 (C_5H_9N, 10), 70 (C_4H_8N, 12), 58 (C_3H_8N, 39), 57 (C_3H_7N, 21).

Hydroxypropyllycodine (36).

Hydroxypropyllycodine (5.5 mg) was obtained as a colourless oil from fraction c-4 (18 mg) (scheme 22) by preparative tlc (3% methanol in ether / ammonia vapours); tlc: Rf 0.23 (ethyl acetate); $[\alpha]_D = +11^\circ$ ($\underline{c}$ = 0.39, methanol); uv ($\underline{c}$ = 0.42, ethanol) λ_{max} nm (log ε): 272 (3.7), *ca* 280 (shoulder); ftir υ_{max} cm^{-1}: 3320 (br, NH and OH), 1650 (weak N=C), 1590, 1570; ^1H and ^{13}C nmr: see tables 12 and 13; cims: m/z 301 (100, M^++1); hreims: m/z calcd. for $C_{19}H_{28}N_2O$: 300.2203, found: 300.2199 (19, M^+), 285 ($C_{18}H_{25}N_2O$, 2, M^+-CH_3), 283 ($C_{19}H_{27}N_2$, 0.4, M^+-H_2O), 256 ($C_{17}H_{24}N_2$, 3, M^+-CH_3CHO), 243 ($C_{15}H_{19}N_2$, 100, M^+-C_4H_9), 199 ($C_{13}H_{15}N_2$, 19, M+-C_4H_9,-CH_3CHO), 171 ($C_{11}H_{11}N_2$, 4, M^+-C_4H_9,-CH_3CHO,-C_2H_4).

Acetylation of hydroxypropyllycodine (36).

An impure sample of hydroxypropyllycodine (**36**), Ac_2O (1 mL), and Et_3N (5 drops) were stirred at room temperature overnight. Excess Ac_2O was decomposed by dropwise addition of water, the solvent was evaporated azeotropically with benzene in *vacuo*, and the residue was purified by preparative tlc (silica gel: 1% methanol in ethyl acetate / ammonia vapours; alumina: ethyl acetate:Skellysolve B; 1:4) to afford the diacetate **37** (*ca* 2 mg), tlc: Rf 0.84 (ethyl acetate); ftir υ_{max} cm^{-1}: 1737, 1656, 1242; ^1H and ^{13}C: see tables 12 and 13; cims: m/z 385 (100 M^++1); hreims: m/z calcd. for $C_{23}H_{32}N_2O_3$: 384.2415, found: 384.2405 (71, M^+), 341 ($C_{21}H_{29}N_2O_2$, 100, M^+-

CH_3CHO), 327 ($C_{19}H_{23}N_2O_3$, 13, M^+-C_4H_9), 325 ($C_{21}H_{29}N_2O$, 74, M^+-CH_3CO_2), 324 ($C_{21}H_{28}N_2O$, 26, M^+-CH_3CO_2H), 298 ($C_{19}H_{26}N_2O$, 21, M^+-C_4H_6O), 285 ($C_{17}H_{21}N_2O_2$, 15, M^+-C_4H_9, -C_2H_2O), 267 ($C_{17}H_{19}N_2O$, 58, M^+-C_4H_9,-CH_3CO_2H), 225 ($C_{15}H_{17}N_2$, 35, M^+-C_4H_9,-CH_3CO_2H,-C_2H_2O).

Flabellidine (11), flabelliformine (6c), and acetyldihydrolycopodine (7b).

Fraction d (89 mg) (scheme 22) afforded relatively pure flabellidine (11) (46 mg), flabelliformine (**6c**) (6 mg), and a mixture (7.9 mg) containing acetyldihydrolycopodine (**7b**) and flabelliformine after gradient elution chromatography over alumina (methanol in dichloromethane, 0.5 to 2%). Acetyldihydrolycopodine was identified in the mixture by gc / ms[30] and ^1H nmr.

Flabellidine (11).

Flabellidine was obtained as an opaque colourless solid, tlc: Rf 0.30 (1% methanol in ethyl acetate); ftir υ_{max} cm^{-1}: 3300 (weak, NH), 1660 (-N$\underline{CO}$$CH_3$); ^1H nmr (400 MHz): 3.78 (1H, brs), 3.27-2.91 (2H, brm), 2.75 (1H, brd, J = 11 Hz), 2.43 (1H, brdd, J = 11,11, Hz), 2.09 (3H, s, -NCOC$\underline{H}_3$), 1.17 (1H, ddd, J = 13,13,4 Hz), 0.82 (3H, d, J = 6 Hz, H-16); ^{13}C nmr (100.6 MHz)*: 169.5 (s), 136.7 (s), 56.3 (s), 46.2 (t), 45.4 (t), 44.5 (d), 43.8 (t), 42.5 (t), 34.1 (d), 31.7 (t), 27.2 (t), 26.3 (t), 24.3 (t), 23.6 (q), 22.0 (q), 20.6 (t); hreims[76]: m/z calcd. for $C_{18}H_{28}N_2O$: 288.2203, found: 288.2303 (11, M^+); 231 ($C_{14}H_{19}N_2O$, 100, M^+-C_4H_9), 203 ($C_{12}H_{15}N_2O$, 3, M^+-C_4H_9,-C_2H_4), 189 ($C_{12}H_{17}N_2$, 9, M^+-C_4H_9,-C_2H_2O), 161 ($C_{10}H_{13}N_2$, 4, M^+-C_4H_9,-C_2H_2O, -C_2H_4). Flabellidine was purified further by conversion to its acetyl derivative.

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* Missing one signal in the spectrum.

Acetylation of flabellidine (11).

Flabellidine (30 mg), Ac_2O (2 mL), and Et_3N (5 drops) in dichloromethane (2 mL) were stirred at room temperature overnight. Excess Ac_2O was decomposed with water, the reaction mixture was basified (aq NH_3), and extracted several times with dichloromethane. The dichloromethane extract was concentrated in *vacuo* and purified by column chromatography over alumina (3% methanol in dichloromethane) to afford acetylflabellidine (26.4 mg). Acetylflabellidine crystallizes from ether / acetone as colourless plates, mp: 155-156°C (150-152°C lit[3a]); tlc: Rf 0.59 (ethyl acetate); ftir υ_{max} cm^{-1}: 1651, 1395; uv ($\underline{c}$ = 0.[illegible], methanol) λ_{max} nm (logε): 233 (3.36); ^{1}H nmr (400 MHz): 3.77 (1H, brs), 3.58 (1H, brd, J = 14 Hz), 3.23-3.11 (3H, m), 2.71 (1H, brddd, J = 14,14,2 Hz), 2.33 (1H, m, H-15), 2.12 (3H, s, $-NCOC\underline{H}_3$), 2.10 (3H, s, $-NCOC\underline{H}_3$), 1.18 (1H, ddd, J = 13,13,4 Hz), 0.90 (3H, d, J = 6 Hz, H-16); ^{13}C nmr (100.6 MHz)* : 171.5 (s), 169.6 (s), 136.8 (s), 66.5 (s), 46.1 (t), 43.9 (d), 43.6 (t), 43.0 (2 x t), 34.6 (d), 31.3 (t), 27.4 (t), 27.1 (d), 26.2 (q), 25.6 (t), 24.2 (t), 23.7 (q), 22.4 (q), 22.2 (t); hreims: m/z calcd. for $C_{20}H_{30}N_2O_2$: 330.2309, found: 330.2305 (33, M^+), 273 ($C_{16}H_{21}N_2O_2$, 100, M^+-C_4H_9), 231 ($C_{14}H_{19}N_2O$, 9, M^+-C_4H_9,-C_2H_2O), 203 ($C_{12}H_{15}N_2O$, 3, M^+-C_4H_9,-C_2H_2O,-C_2H_4), 189 ($C_{12}H_{17}N_2$, 5, M^+-C_4H_9,-$2C_2H_2O$), 161 ($C_{10}H_{13}N_2$, 3, M^+-C_4H_9,-$2C_2H_2O$,-C_2H_4).

Flabelliformine (6c).

Flabelliformine (**6c**) crystallizes from acetone as a white powder, mp: 204-206°C (210-211°C lit[3a]); tlc: Rf 0.2 (2% methanol in dichloromethane); ftir υ_{max} cm^{-1}: 3400, 1709, 1074, ^{1}H nmr (400 MHz): 3.74 (1H, ddd, J = 11,11,4 Hz), 3.40 (1H, ddd, J =

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* Missing one signal in the spectrum.

14,14,4 Hz), 3.26 (1H, ddd, J = 14,4,2 Hz), 2.46 (1H, ddd, J = 14,14,5 Hz); 1.28 (1H, dddd, J = 13,13,4,2 Hz), 0.93 (1H, dd, J = 13,13 Hz), 0.81 (3H, d, J = 7 Hz); ^{13}C nmr (100.6 MHz)^{76}: 211.0 (s, C-5), 80.0 (s, C-4), 59.1 (s, C-13), 49.2 (t, C-9), 46.3 (t, C-1), 46.0 (d, C-12), 44.8 (t, C-14), 42.8 (t, C-8), 39.5 (t, C-6), 36.7 (d, C-7), 27.5 (t, C-11)*, 25.8 (t, C-10)*, 25.5 (d, C-15), 23.2 (q, C-16), 17.0 (t, C-2); hreims: m/z calcd. for $C_{16}H_{25}NO_2$: 263.1887, found: 263.1878 (75, M$^+$), 246 ($C_{16}H_{24}NO$, 10, M$^+$-OH), 206 ($C_{12}H_{16}NO_2$, 100, M$^+$-C_4H_9), 178 ($C_{11}H_{16}NO$, 5, M$^+$-C_4H_9,-CO), 164 ($C_{10}H_{14}NO$, 6, M$^+$-C_4H_9,-C_2H_2O), 160 ($C_{11}H_{14}N$, 6, M$^+$-C_4H_9,-CO,-H_2O).

B3.3. Isolation of compounds 12c, 27, and 29.

Fraction E (203 mg) (table 28) was separated by gradient elution chromatography over alumina (methanol in dichloromethane, 0.5% to 3%) into 5 fractions **a** to **e**. The fractions **d** (62 mg) and **e** (39 mg) were further purified.

Lobscurinol (27).

Lobscurinol (**27**) (2 mg) was obtained as a colourless oil from fraction **d** (62 mg) by gradient elution chromatography (alumina, methanol in dichloromethane, 0.5% to 1%) and further purification by two successive preparative tlc (methanol in dichloromethane 3% and 5% under an atmosphere of ammonia), tlc: Rf 0.11 (3% methanol in ethyl acetate); ftir υ_{max} cm^{-1}: 3600-3200 (br, OH), 1658; ^{1}H and ^{13}C nmr: see tables 7 and 8; hreims: m/z calcd. for $C_{17}H_{25}NO_2$: 275.1887, found: 275.1881 (17, M$^+$), 274 ($C_{17}H_{24}NO_2$, 3, M$^+$-H), 258 ($C_{17}H_{24}NO$, 12, M$^+$-OH), 247 ($C_{16}H_{25}NO$, 4, M$^+$-CO), 232 ($C_{15}H_{22}NO$, 4, M$^+$-CH_3,-CO), 192 ($C_{12}H_{18}NO$, 20, M$^+$-C_5H_7O), 174 ($C_{12}H_{16}N$,

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* Signals may be reversed.

6, M^+-C_5H_7O,-H_2O), 85 ($C_5H_{11}N$, 21), 84 ($C_5H_{10}N$, 100), 83 (C_5H_9N, 22), 71 (C_4H_9N, 6), 70 (C_4H_8N, 15), 58(C_3H_8N, 28), 57 (C_3H_7N, 44).

Des-N-methyl-α-obscurine (12c).

A uv active material (1.3 mg), showing a single spot by tlc was obtained from chromatography of fraction **d** above and was identified as a mixture of lobscurinol and des-N-methyl α-obscurine (gc / ms). The mixture was acetylated (excess Ac_2O, 5 drops Et_3N, overnight). The reaction mixture was concentrated in *vacuo* and separated by preparative tlc (3% methanol in dichloromethane) to afford acetyllobscurinol (**30**) and acetyl des-N-methyl-α-obscurine (**12c**), tlc: Rf 0.49 (3% methanol in dichloromethane/ammonia vapours, silica gel); ^{1}H nmr (400 MHz): 6.49 (1H, brs $W_{1/2}$ = 9 Hz, N$\underline{H}$), 3.64 (1H, brd, J = 13.5 Hz), 3.14 (1H, brd, J = 10 Hz), 2.70 (1H, brddd, J = 13.5,12,3 Hz), 2.13 (3H, s, -NCOC$\underline{H}_3$), 2.08 (1H, m, H-15), 1.92 (1H, brs, $W_{1/2}$ = 14 Hz), 0.91 (3H, d, J = 6.5 Hz); hreims[76]: m/z calcd. for $C_{18}H_{26}N_2O_2$: 302.1996, found: 302.1997 (39, M^+), 259 ($C_{16}H_{23}N_2O$, 6, M^+-CH_3CO), 245 ($C_{14}H_{17}N_2O_2$, 100, M^+-C_4H_9), 203 ($C_{12}H_{15}N_2O$, 18, M^+-C_4H_9,-C_2H_2O), 175 ($C_{10}H_{11}N_2O$, 4, M^+-C_4H_9,-C_2H_2O,-C_2H_4).

Epilobscurinol (29).

Relatively pure epilobscurinol (**29**) (1 mg) was obtained from fraction **e** (30 mg) by gradient elution chromatography (alumina, methanol in dichloromethane, 0.5 to 2%) and three successive purifications by preparative tlc (5% methanol in dichloromethane / ammonia vapours). Epilobscurinol was purified further by conversion to its acetyl derivative **31**[*]. The crude acetylation product was purified by preparative tlc (3% methanol in dichloromethane / ammonia vapours), tlc: Rf 0.29 (3% methanol in ethyl acetate) or 0.78 (5% methanol in ether / ammonia vapours, silica gel); ^{1}H nmr: see table 9; hreims: m/z calcd. for $C_{19}H_{27}NO_3$: 317.1992, found: 317.1991 (47, M^+), 302 ($C_{18}H_{24}NO_3$, 5, M^+-CH_3), 274 ($C_{17}H_{24}NO_2$, 13, M^+-CO, -CH_3), 258 ($C_{17}H_{24}NO$, 68, M^+-CH_3CO_2), 229 ($C_{16}H_{23}N$, 2, M^+-CH_3CO_2H,-CO), 215 ($C_{15}H_{19}$, 23, M^+-$C_4H_8NO_2$), 85 ($C_5H_{11}N$, 20), 84 ($C_5H_{10}N$, 100), 83 (C_5H_9N, 21), 82 (C_5H_8N, 6), 71 (C_4H_9N, 5), 70 (C_4H_8N, 25), 58 (C_3H_8N, 57), 57 (C_2H_7N, 37).

Oxidation of lobscurinol (27) to lobscurinine (28) and conversion of lobscurinine to obscurinine (13).

Lobscurinol (1.8 mg) dissolved in dichloromethane (0.2 mL) was added to a suspension of pyridinium chlorochromate (5 mg, 0.02 mmol) in dichloromethane (0.4 mL) at 0°C. The mixture was stirred at 0°C for 30 minutes, then warmed to room temperature for 3 hrs. The reaction mixture was filtered through a pipette containing alumina and washed with chloroform. The organic solution was evaporated under reduced pressure to afford lobscurinine (**28**) (1 mg), tlc: Rf 0.65 (ethyl acetate); mp: unavailable; ftir υ_{max} cm^{-1}: 1722, 1663, 1643; ^{1}H nmr (400 MHz): 6.95 (1H, dd, J = 11.7,7.3 Hz, H-3), 5.92 (1H, brs, $W_{1/2}$ = 5.6 Hz, H-14), 3.33 (1H, dddd; J = 14,11.7,11.7,2 Hz, H-2), 2.35 (3H, s, $-NC\underline{H}_3$), 2.23 (1H, d, J = 17.3 Hz, H-8β), 1.98 (3H, s, H-16), 1.83 (1H, ddd, J = 15.22,6.6,2.5 Hz); ^{13}C nmr: see table 8; cims: m/z 274 (100, M^++1); hreims: m/z calcd. for $C_{17}H_{23}NO_2$: 273.1730, found: 273.1721 (13, M^+), 258 ($C_{16}H_{20}NO_2$, 34, M^+-CH_3), 190 ($C_{12}H_{16}NO$, 5, M^+-C_5H_7O), 85 ($C_5H_{11}N$,

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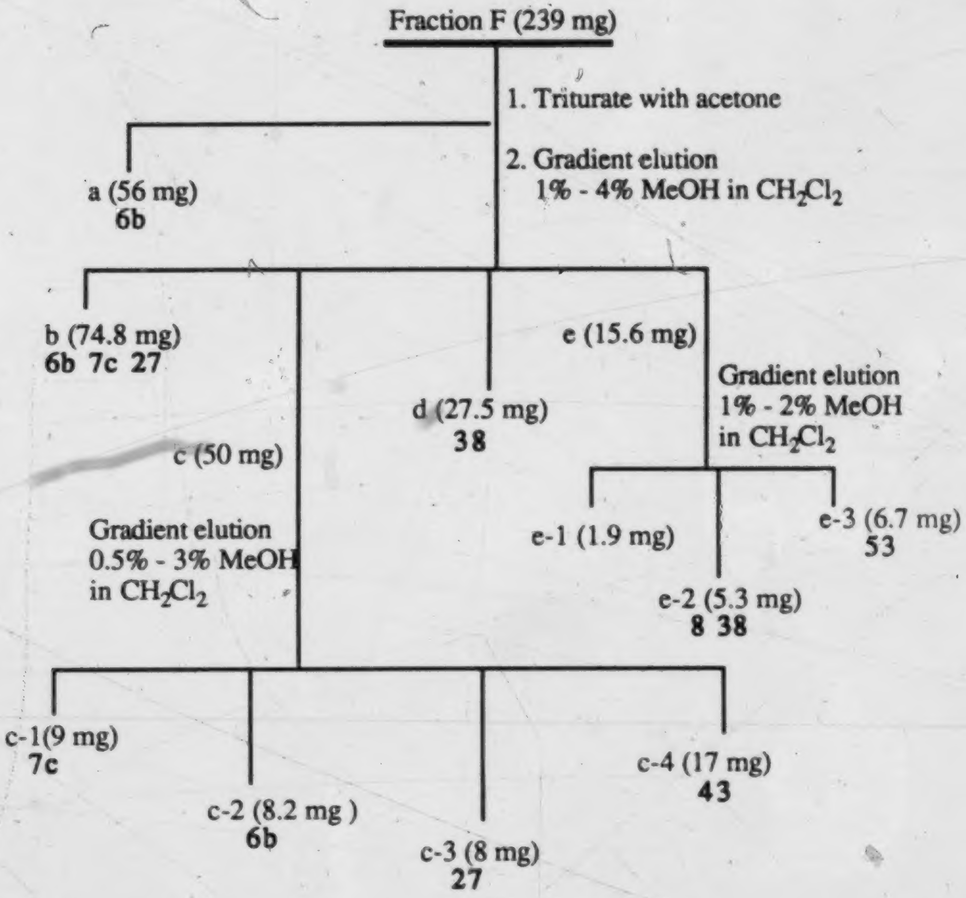
[*] Unless otherwise specified, all acetylations were performed in excess Ac_2O and pyridine (1 mL). The reaction mixture was concentrated azeotropically with benzene in *vacuo*.

20), 84 ($C_5H_{10}N$, 100), 70 (C_4H_8, 14), 57 (C_3H_7, 50). A few drops of aqueous NH_3 was added to lobscurinine (**28**) dissolved in a minimum amount of chloroform. The mixture was allowed to stand at room temperature overnight. Concentration under reduced pressure gave a single compound, identified as obscurinine (**13**), in quantitative yield.


Fraction F (239 mg)
1. Triturate with acetone
2. Gradient elution 1% - 4% MeOH in CH_2Cl_2
a (56 mg)
6b
b (74.8 mg)
6b 7c 27
c (50 mg)
Gradient elution 0.5% - 3% MeOH in CH_2Cl_2
d (27.5 mg)
38
e (15.6 mg)
Gradient elution 1% - 2% MeOH in CH_2Cl_2
e-1 (1.9 mg)
e-2 (5.3 mg)
8 38
e-3 (6.7 mg)
53
c-1(9 mg)
7c
c-2 (8.2 mg)
6b
c-3 (8 mg)
27
c-4 (17 mg)
43

Scheme 23. Isolation of clavolonine (**6b**), β-lofoline (**7c**), lycofoline (**8**), lyconnotinol (**38**), and acrifolinol (**43**).

B3.4. Isolation of compounds 6b, 7c, 8, 38, and 43 .

Clavolonine (**6b**) (56 mg) was precipitated upon addition of cold acetone to fraction **F** (239 mg) (table 28). The residue (183 mg) was separated by gradient elution chromatography over alumina (methanol in dichloromethane, 1 to 4%) into 4 fractions **b** to **e** as shown in scheme 23. Each of fractions **c**, **d**, and **e** was further fractionated.

Clavolonine (6b).

Clavolonine crystallizes from acetone-ether as colourless needles, mp: 202-204°C (238°C lit[3a]); tlc: Rf 0.36 (2% methanol in dichloromethane); $[\alpha]_D = +139$ (c = 0.31 methanol); ftir υ_{max} cm^{-1}: 3220, 1692, 1060, 1040; ^{1}H nmr (400 MHz): 3.37 (1H, dd, J = 10,4.5 Hz, H-8ax), 3.27 (1H, ddd, J = 14,14,4 Hz, H-1ax), 3.11 (1H, ddd, J = 12.5,12.5,3 Hz, H-9ax), 2.89 (1H, brdd, J = 12,3 Hz, H-4ax), 2.60 (3H, m, H-6eq,H-9eq,H-14eq), 2.50 (1H, brdd, J = 14,5 Hz, H-1eq), 2.30 (1H, brdd, J = 15,6 Hz, H-6ax), 2.11 (1H, ddd, J = 6,4.5,2 Hz, H-7eq), 2.03 (1H, brd, J = 14.5 Hz, H-3), 1.89-1.63 (5H, m, 2H-10,H-2,H-11,OH), 1.62-1.51 (3H, m, H-2,H-3,H-11), 1.33 (1H, m, H-3), 1.26 (1H, m, H-15), 0.98 (1H, dd, J = 13,13 Hz, H-14ax), 0.96 (3H, d, J = 6 Hz, H-16); ^{13}C nmr (100.6 MHz)[75]: 213.0 (s, C-5), 79.0 (d, C-8), 59.6 (s, C-13), 47.2 (t, C-9), 47.1 (t, C-1), 43.9 (d, C-12), 42.9 (d, C-7)*, 42.8 (d, C-4)*, 41.9 (t, C-14), 36.4 (t, C-6), 32.9 (d, C-15), 26.1 (t, C-10), 25.0 (t, C-11), 19.3 (t, C-3), 19.4 (q, C-16), 18.7 (t, C-2); hreims: m/z calcd. for $C_{16}H_{25}NO_2$: 263.1887, found: 263.1883 (5, M$^+$), 190 ($C_{12}H_{16}NO$, 100, M$^+$-C_4H_9O), 162 ($C_{11}H_{16}N$, 3, M$^+$-C_4H_9O,-CO), 134($C_9H_{12}N$, 2, M$^+$-C_4H_9O,-CO,-C_2H_4).

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* Signals may be reversed.

β-Lofoline (7c).

β-Lofoline (9 mg) (fraction **c-1**, scheme 23) was obtained as a colourless opaque solid, tlc: Rf 0.09 (2% methanol in dichloromethane); ftir υ_{max} cm^{-1}: 3400, 3150, 1734, 1291, 1262, 1246, 1233, 1106, 1066, 1053, 1035; ^{1}H nmr (400 MHz): 5.06 (1H, ddd, J = 6,6,1.5 Hz, H-5eq), 3.34 (1H, dd, J = 9,5 Hz, H-8ax), 3.33 (1H, ddd, J = 14,14,5 Hz, H-1ax), 3.13 (1H, ddd, J = 12,12,3 Hz, H-9ax); 2.64 (1H, dd, J = 14,6 Hz, H-14eq), 2.53-2.39 (4H, m), 2.02 (3H, s, $C\underline{H}_3CO_2$-), 1.98-1.59 (9H, m), 1.43-1.23 (4H, m), 1.04 (3H, d, J = 6 Hz, H-16), 0.91 (1H, dd, J = 13,13 Hz, H-14ax); ^{13}C nmr (100.6 MHz): 170.4 (s, CO) 78.9 (d, C-8), 69.7 (d, C-5), 54.4 (s, C-13), 47.5 (t, C-9), 46.7 (t, C-1), 43.6 (d, C-7), 41.6 (t, C-14), 41.3 (d, C-12), 32.0 (d, C-4), 31.0 (d, C-15), 26.3 (t), 24.4 (t, 2 x C), 22.7 (t), 21.5 (q), 20.8 (q), 20.0 (t); hreims: m/z calcd. for $C_{18}H_{29}NO_3$: 307.2149, found: 307.2146 (8, M^+), 248 ($C_{16}H_{26}NO$, 6, M^+-CH_3CO_2), 234 ($C_{14}H_{20}NO_2$, 100, M^+-C_4H_9O), 174 ($C_{12}H_{16}N$, 47, M^+-C_4H_9O,-CH_3CO_2H), 146 ($C_{10}H_{12}N$, 12, M^+-C_4H_9O, -CH_3CO_2H,-C_2H_4). β-lofoline was identified by comparison with an authentic sample (tlc, hreims, ^{1}H nmr).

Acrifolinol (43).

Acrifolinol (*ca* 3.8 mg) was obtained from fraction **c-4** (17 mg) (scheme 23) by chromatography over alumina (2% methanol in ethyl acetate), tlc: Rf 0.46 (5% methanol in ether / ammonia vapours, silica gel); $[\alpha]_D = -20^o$ ($\underline{c}$ = 0.47, methanol); ftir υ_{max} cm^{-1}: 3240 (br, OH), 1052, 989, 752; ^{1}H nmr (400 MHz): 5.44 (1H, t, J = 4 Hz, H-11), 4.01 (1H, ddd, J = 10,4.5,1 Hz, H-8eq), 3.84 (1H, brs, $W_{1/2}$ = 10 Hz, H-5eq), 3.42 (1H, brs, OH), 3.03 (1H, brdd, J = 12,12 Hz, H-1ax), 2.90 (1H, ddd, J = 10,5.5,2 Hz, H-7eq), 2.82 (1H, ddd, J = 12,6,6 Hz, H-9ax), 2.59 (1H, brd, J = 12 Hz, H-1eq), 2.54 (1H, ddd, J = 12,5,1 Hz, H-9eq), 2.34 (1H, ddd, J = 15,2,2 Hz, H-6eq), 2.19 (1H, dd, J = 14,14 Hz, H-14ax), 2.19-2.09 (2H, m, 2H-10), 2.01-1.96 (5H, m, H-15,H-2,2H-3,H-4), 1.77 (1H, ddd, J = 15,5.5,5.5 Hz, H-6ax), 1.50 (1H, dd, J = 14,6 Hz, H-14eq), 1.46 (1H, brd, J = 12 Hz, H-2), 0.96 (3H, d, J = 7 Hz, H-16); ^{13}C nmr: see table 18; cims: m/z 264 (55, M^{+}+1), 246 (100, M^{+}-H_2O +1); hreims: m/z calcd. for $C_{16}H_{25}NO_2$: 263.1887, found: 263.1884 (100, M^{+}), 262 ($C_{16}H_{24}NO_2$, 30, M^{+}-H), 246 ($C_{16}H_{24}NO$, 41, M^{+}-OH), 245 ($C_{16}H_{23}NO$, 5, M^{+}-H_2O), 204 ($C_{13}H_{18}NO$, 30, M^{+}-C_3H_7O), 192 ($C_{12}H_{18}NO$, 84, M^{+}-C_4H_7O), 191 ($C_{12}H_{17}NO$, 36, M^{+}-C_4H_8O), 190 ($C_{12}H_{16}NO$, 27, M^{+}-C_4H_9O).

Lyconnotinol (38).

A 1:1 mixture (by ^{1}H nmr, 3 mg) of lyconnotinol and an unidentified alkaloid was obtained from fraction d (27 mg) (scheme 23) by gradient elution chromatography over alumina (1% to 2% methanol in dichloromethane) followed by preparative tlc (3% methanol in dichloromethane / ammonia vapours). The mixture was acetylated (excess Ac_2O, room temperature, overnight), concentrated in *vacuo*, and purified by filtration over alumina (pipette) and washing with ethyl acetate. The ethyl acetate extract afforded monoacetyllyconnotinol (39) upon concentration in *vacuo*, tlc: Rf 0.60 (Skellysolve B:ethyl acetate; 1:1); mp: 192-193°C (methiodide)[*]; ftir υ_{max} cm^{-1}: 3200 (OH), 2810, 2720, 2660 (*Bohlmann bands*), 1736, 1247, 1214, 1162, 1097, 1060, 1033; uv (c = .015, ethanol, methiodide) λ_{max} nm (log ε): 234 (3.72); ^{1}H nmr (400 MHz): 6.20 (1H, brd, J = 10 Hz, H-11), 5.68 (1H, brddd, J = 10,5,2 Hz, H-10), 5.49 (1H, t, J = 4 Hz, H-7), 4.10 (1H, ddd, 7.5,3.4,3.3 Hz, H-5eq), 3.87 (1H, dd, J = 10.6,5.2 Hz, H-8a), 3.71 (1H, dd, J = 10.5,7 Hz, H-8b), 3.20 (1H, brd, J = 18 Hz, H-9eq), 3.09 (1H, brdd, J = 18,5 Hz, H-9ax), 2.79 (1H, brddd, J = 20,7.5,4 Hz, H-6ax), 2.68 (1H, ddd, J = 11.5,11.5,4.4 Hz, H-1ax), 2.58 (1H, dt, J = 11.5,2,2 Hz, H-1eq), 2.27 (1H, brd, J = 20.4 Hz, H-6eq), 2.04 (3H, s, $C\underline{H}_3CO_2$-), 1.98 (3H, m, H-15), 1.80-1.66 (6H, m), 1.52 (1H, brdm, J = 15 Hz, H-3a), 1.38 (1H, brd, 3.3, -O$\underline{H}$), 0.89 (1H, d, J = 7 Hz): ^{13}C nmr: see table 16; hreims: m/z calcd. for $C_{18}H_{27}NO_3$: 305.1992, found: 305.1988 (0.6, M^+), 190 ($C_{12}H_{16}NO$, 100, M^+-$C_6H_{11}O_2$), 172 ($C_{12}H_{14}N$, 3, M^+-$C_6H_{11}O_2$, -H_2O), 144 ($C_{10}H_{10}N$, 3, M^+-$C_6H_{11}O_2$,-H_2O,-C_2H_4).

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[*] An excess solution of methyl iodide was added to a solution of the base in minimum amount of acetone. The solvent was evaporated under the fume hood to afford a crystalline methiodide salt of the base, which was recrystallized from acetone.

Reduction of lyconnotine (41) to lyconnotinol (38).

Lyconnotine (41), (119 mg) and $LiAH_4$ (117 mg) in dry ether (10 mL) were heated under reflux for 4 hrs. Excess $LiAlH_4$ was decomposed with acetic acid, the mixture was diluted with ether and filtered through Whatman filter paper. The filtrate was diluted with water (5 mL), the ether and aqueous layers were separated, and the aqueous layer was extracted with chloroform (3 x 10 mL). The combined ether and chloroform extracts were concentrated in *vacuo*, filtered through alumina, and washed with 2% methanol in chloroform to afford lyconnotinol (38) (100 mg, 93%). Lyconnotinol crystallizes from methanol / acetone as offwhite prisms, mp: 173-175°C; tlc: Rf 0.45 (1% methanol in ethyl acetate); $[\alpha]_D = +103^\circ$ ($\underline{c}$ = 0.43, methanol); uv ($\underline{c}$ = 0.015, ethanol) λ_{max} nm ($\log\varepsilon$): 235 (3.72); ftir υ_{max} cm^{-1}: 3340 (br, OH), 3027, 3013, 3006 (olefinic CH), 2880, 2865, 2824, 2894, 2789, 2773, 2768, 2763, 2747, 2742 (*Bohlmann bands*), 1642, 1099, 1081, 1062, 1033; ^1H nmr (400 MHz): 6.19 (1H, brd, J = 10 Hz, H-11), 5.71 (1H, brdd, J = 10,5 Hz, H-10), 5.49 (1H, t, J = 4 Hz, H-7), 4.09 (1H, brs, $W_{1/2}$ = 16 Hz, H-5eq), 3.42 (1H, dd, J = 10.4,5 Hz, H-8a), 3.28 (1H, dd, J = 10.4,6.3 Hz, H-8b), 3.23 (1H, brd, J = 18 Hz, H-9eq), 3.12 (1H, brdd, J = 18,5 Hz, H-9ax), 2.80 (1H, brdd, J = 20,7,4 Hz, H-6ax), 2.74 (1H, ddd, J = 12,12,4.5 Hz, H-1ax), 2.58 (1H, brd, J = 12 Hz, H-1eq), 2.27 (1H, brd, J = 12 Hz, H-6eq), 1.93-1.66

(7H, m), 1.52 (1H, brdm, J = 15 Hz, H-3a), 1.45 (1H, brs, OH), 0.90 (3H, d, J = 6.4 Hz, H-16); ^{13}C nmr: see table 16; cims: m/z 264 (100, M^{+}+1); hreims: m/z calcd. for $C_{16}H_{25}NO_2$: 263.1887, 190 ($C_{12}H_{16}NO$, 100, M^{+}-C_4H_9O), 172 ($C_{12}H_{14}N$, 3, M^{+}-H_2O), 144 ($C_{10}H_{10}N$, 3, M^{+}-C_4H_9O,-H_2O).

Acetylation of synthetic lyconnotinol (38).

Lyconnotinol (84 mg, 0.3194 mmol), Ac_2O (10 mL), and Et_3N (5 drops) were stirred at room temperature for 15 hrs. Excess Ac_2O was decomposed with water and most of the solvent was removed by azeotropic concentration with benzene in *vacuo*. The mixture was diluted with water (10 mL), made basic (10% NaOH), and extracted with chloroform (5 x 20 mL). The organic solution was concentrated in *vacuo* and separated by chromatography over alumina (Skellysolve B:ethyl acetate; 1:1) into 3 fractions **a** to **c** containing diacetyllyconnotinol (**40**) (8.1 mg), a mixture of mono and diacetyllyconnotinol (57.7 mg) and monoacetyllyconnotinol (18 mg), respectively. Fraction **b** was separated by preparative tlc (3% methanol in ethyl acetate / ammonia vapours) to afford **39** and **42** (50 mg and 3.8 mg, respectively). Mono and diacetyllyconnotinol was each converted to the corresponding methiodide as described previously. Monoacetyllyconnotinol is identical with the sample from natural sources (see p.153 for spectral data).

Synthetic diacetyllyconnotinol (42).

tlc: Rf 0.78 (Skellysolve B:ethyl acetate; 1:1); mp 176-177°C (methiodide, acetone-ether); ftir υ_{max} cm^{-1}: 2800, 2720, 2660 (*Bohlmann bands*), 1737, 1248, 1238, 1086, 1061, 1034 ; ^{1}H nmr (400 MHz): 6.17 (1H, brd, J = 10 Hz, H-11), 5.70 (1H, brdd, J = 10,5 Hz), 5.48 (1H, t, J = 4 Hz, H-7), 5.17 (1H, dd, J = 7.9,4.2 Hz, H-5eq), 3.87 (1H, dd, J = 10.7,5.2 Hz, H-8a), 3.72 (1H, dd, J = 10.7,6.8 Hz, H-8b), 3.19 (1H, brd, J = 19 Hz, H-9eq), 3.10 (1H, brdd, J = 19,5 Hz, H-9ax), 2.86 (1H, ddd, J = 21,7.9,5 Hz, H-6ax), 2.64 (1H, ddd, J = 12,12,4 Hz, H-1ax), 2.57 (1H, brd, J = 12 Hz, H-1eq), 2.21 (1H, brd, J = 21 Hz, H-6eq), 2.06 (3H, s, $C\underline{H}_3CO_2$-), 2.05 (3H, s, $C\underline{H}_3CO_2$-), 1.94-1.84 (2H, m), 1.79-1.63 (5H, m), 0.92 (3H, d, J = 6.7 Hz, H-16); ^{13}C nmr: see table 16.

Lycofoline (8)

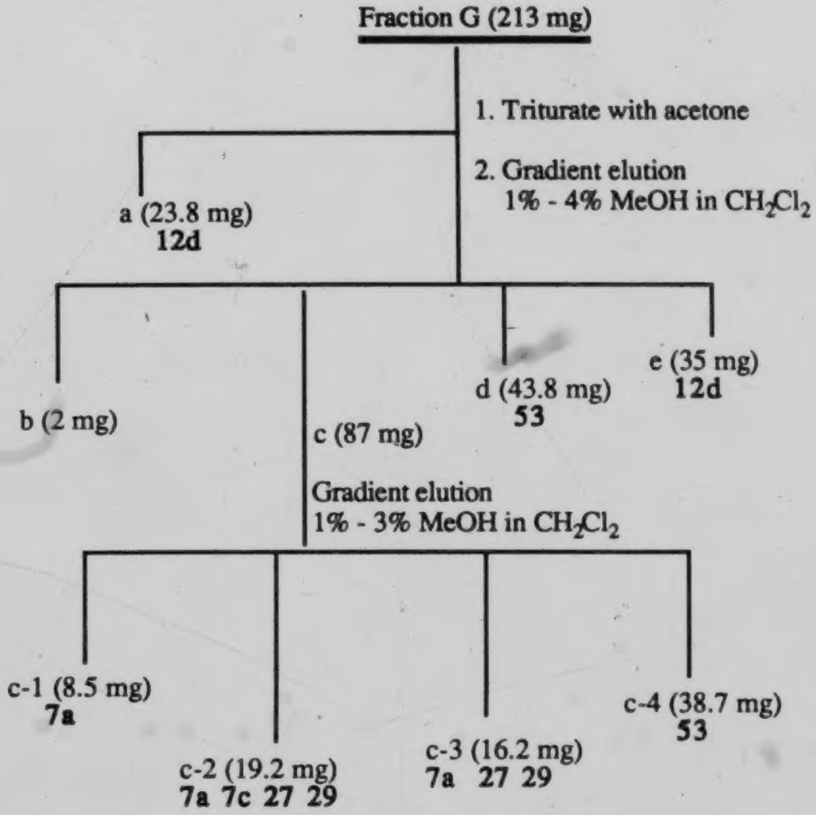
Lycofoline (1 mg) was obtained from fraction **e-2** (15.6 mg) (scheme 23) by pipette column chromatography over alumina (methanol in dichloromethane, 0.5 to 2 %), tlc: Rf 0.36 (5% methanol in ether / ammonia vapours, silica gel); ftir υ_{max} cm^{-1}: 3380 (br, OH), 1665, 1089, 1060, 1055, 1043, 1024, 1007, 756; ^{1}H nmr (400 MHz): 5.44 (1H, dd, J = 5, 3.5 Hz H-11), 3.95 (1H, brddd, J = 6,6,1 Hz, H-5 eq), 3.23 (1H, dd, J = 10, 5.5 Hz, H-8ax), 2.95 (1H, ddd, J = 12,12,4 Hz, H-1ax), 2.7 (1H, m, H-15ax), 2.65 (2H, m, 2H-9), 2.53 (2H, m, H-7eq,H-1eq), 2.23 (1H, m, H-10eq), 2.21 (1H, dd, J = 13,6.5 Hz, H-14eq), 2.11 (1H, brd, J = 15.5 Hz, H-6eq), 2.02 (1H, ddd, J = 9,9,4 Hz, H-10ax), 1.94 (1H, m, H-4), 1.89 (1H, ddd, J = 15.5,6,6 Hz, H-6 ax), 1.81-1.73 (2H, m, H-2,H-3), 1.65-1.50 (m, H-2 and / or OH), 1.47 (1H, m, H-3), 1.06 (1H, dd, J = 13,13 Hz, H-14 ax), 1.01 (3H, d, J = 7 Hz, H-16); ^{13}C nmr: see table 18; cims: m/z 264 (55, M^++1), 246 (100, (M^+-H_2O)+1); hreims: m/z calcd. for $C_{16}H_{25}NO_2$: 263.1887, found: 263.1881 (100, M^+), 262 ($C_{16}H_{24}NO_2$, 32, M^+-H), 246 ($C_{16}H_{24}NO$, 59, M^+-OH), 245 ($C_{16}H_{23}NO$, 6, M^+-H_2O), 230 ($C_{15}H_{20}NO$,8, M^+-CH_3,-H_2O), 219 ($C_{14}H_{21}NO$, 13, M^+-C_2H_4O), 204 ($C_{13}H_{18}NO$, 37, M^+-C_3H_7O), 192 ($C_{12}H_{18}NO$, 93, M^+-C_4H_7O), 191 ($C_{12}H_{17}NO$, 34, M^+-C_4H_8O), 190 ($C_{12}H_{16}NO$, 33, M^+-C_4H_9O), 188 ($C_{13}H_{18}N$, 8, M^+-C_3H_5O,-H_2O), 174 ($C_{12}H_{16}N$, 15, M^+-C_4H_9O,-H_2O), 146 ($C_{10}H_{12}N$, 8, M^+-$C_4H_9O_2$,-C_2H_4).

Preparation of acrifolinol (43) and lycofoline (8) from acrifoline (44).

NaOH (97%, 0.5042 g) dissolved in ethanol (98%):water; 10:2 (10 mL) was added to a mixture of acrifoline hydrobromide (56.5 mg) and $NaBH_4$ (123 mg). The mixture was allowed to stand at room temperature for 30 minutes then heated under reflux for 2 hrs. Excess $NaBH_4$ was decomposed by dropwise addition of acetic acid and most of the ethanol was evaporated under reduced pressure. The residue was diluted with water (20 mL), made basic (10% NaOH), and extracted several times with chloroform. The chloroform extract was washed twice with brine, dried ($MgSO_4$), and concentrated in *vacuo* to afford crude reaction product, two components of lower Rf than starting material, in quantitative yield. The crude reaction product afforded acrifoline (43) (19.1 mg) and a mixture containing mainly lycofoline (8) (6.2 mg) by preparative tlc (5% methanol in ether / ammonia vapours). Lycofoline (5 mg) was obtained from the mixture by further preparative tlc (same chromatographic condition as above).

B3.5. Isolation of compounds 7c, 12d, and 53.

Fraction **a** (23.8 mg) containing mainly des-N-methyl-β-obscurine (12d) was precipitated by addition of cold acetone to fraction **G** (213 mg) (table 28). The residue (*ca* 184 mg) was separated by gradient elution chromatography over alumina (methanol in dichloromethane, 1% to 4%) into 4 fractions **b** to **e** (scheme 24). Fraction **b** was separated further.


Fraction G (213 mg)
1. Triturate with acetone
2. Gradient elution
1% - 4% MeOH in CH$_2$Cl$_2$
a (23.8 mg)
12d
b (2 mg)
c (87 mg)
d (43.8 mg)
53
e (35 mg)
12d
Gradient elution
1% - 3% MeOH in CH$_2$Cl$_2$
c-1 (8.5 mg)
7a
c-2 (19.2 mg)
7a 7c 27 29
c-3 (16.2 mg)
7a 27 29
c-4 (38.7 mg)
53

Scheme 24. Isolation of dihydrolycopodine (**7a**), des-N-methyl-β-obscurine (**12d**), and unidentified alkaloid (**53**).

Des-N-methyl-β-obscurine (12d).

Des-N-methyl-β-obscurine (*ca* 17 mg) was obtained from fraction **a** (23.8 mg) by flash column chromatography (3% methanol in dichloromethane presaturated with ammonia). Des-N-methyl-β-obscurine crystallizes from methanol-acetone as colourless needles, mp: 290-292.5°C (313°C lit[16b]); tlc: Rf 0.42 (5% methanol in dichloromethane); ftir υ_{max} cm^{-1}: 3302, 3021, 1659, 1623, 1554; $[\alpha]_D = -34°$ (c = 0.21, methanol); uv (c =

0.8, methanol) λ_{max} nm (log ε): 203 (3.9), 230 (3.8), 314 (3.7); ^{1}H nmr (400 MHz): 7.57 (1H, d, J = 9 Hz, H-3), 6.44 (1H, d, J = 9 Hz, H-2), 2.91 (1H, brdd, J = 19,7 Hz, H-6ax), 2.76 (1H, dm, J = 13 Hz, H-9eq), 2.43 (1H, brddd, J = 13,13,2 Hz, H-9ax), 2.40 (1H, brd, J = 19 Hz, H-6eq), 2.10 (1H, brsext, J = 3 Hz, H-7eq), 1.70 (1H, brdm, J = 12 Hz, H-8eq), 1.60-1.35 (5H, m, H-12,H-14eq,2H-10,H-15), 1.30-1.20 (3H, m, H-8ax,2H-11), 1.03 (1H, dd, J = 11,11 Hz, H-14ax), 0.80 (3H, d, J = 6.5 Hz, H-16); ^{13}C nmr: see table 13; hreims: m/z calcd. for $C_{16}H_{22}N_2O$: 258.1734, found: 258.1733 (14, M^+), 201 ($C_{12}H_{13}N_2O$, 100, M^+-C_4H_9), 173 ($C_{10}H_9N_2O$, 13, M^+-C_4H_9,-C_2H_4).

Dihydrolycopodine (7a) and unidentified alkaloid 53.

Dihydrolycopodine (**7a**) (8.5 mg) and an unidentified alkaloid (**53**) (38.7 mg) were obtained from fraction **c** (87 mg) (scheme 24) by gradient elution chromatography over alumina (methanol in dichloromethane, 1 to 2 %).

Dihydrolycopodine (7a).

Tlc: Rf 0.24 (3% methanol in chloroform); ftir υ_{max} cm^{-1}: 3160, 1114, 991, ^{1}H nmr (400 MHz): 3.95 (1H, ddd, J = 6.5, 6.5,2 Hz, H-5eq), 3.45 (1H, ddd, J = 14,14,4 Hz, H-1ax), 3.16 (1H, ddd, J = 12,12,3 Hz, H-9ax), 2.85 (1H, ddddq, J = 13,13,6,6,6 Hz, H-15ax), 2.62 (1H, dd, J = 13,6 Hz, H-14eq), 2.56-2.47 (2H, m, H-1eq,H-9eq), 2.33 (1H, brddd, J = 14,5,3 Hz, H-4ax), 2.08 (1H, ddd, J = 14,6,6 Hz, H-6ax), 1.99 (1H, ddddd, J = 13,13,13,4.5,4.5 Hz, H-2ax), 1.82 (1H, dddd, J = 13,13,13,5 Hz, H-3ax), 1.21 (1H, brddd, J = 12.5,12.5,5 Hz, H-8ax), 0.87 (3H, d, J = 6 Hz, H-16), 0.77 (1H, dd, J = 13,13 Hz, H-14ax); ^{13}C nmr (74.0 MHz)[75]: 68.5 (d, C-5), 55.2 (s, C-13), 47.2 (t, C-9), 46.9 (t, C-1), 45.6 (d), 42.9 (t), 41.8 (t), 35.4 (d), 33.6 (t), 32.5

(d), 26.4 (t), 24.7 (t), 24.0 (d), 23.3 (q, C-16), 23.3 (t), 20.5 (t); **hreims**: m/z calcd. for $C_{16}H_{27}NO$: 249.2092, found: 249.2084 (7, M^+), 232 ($C_{16}H_{26}N$, 2, M^+-OH), 192 ($C_{12}H_{18}NO$, 100, M^+-C_4H_9), 174 ($C_{12}H_{16}N$, 3, M^+-C_4H_9,-H_2O), 148 ($C_{10}H_{14}N$, 2, M^+-C_4H_9,-H_2O, -C_2H_4).

Unidentified base (53).

Base **53** was obtained as an opaque solid, which was converted to its perchlorate (60 % aq $HClO_4$)*, mp: 195-223°C**; tlc: Rf 0.36 (3% methanol in chloroform); ftir υ_{max} cm^{-1}: 1704; ^{1}H nmr (400 MHz): 3.95 (1H, ddd, J = 13,13,3 Hz), 3.63 (1H, ddd, J = 14,14,5 Hz), 3.10 (1H, brd, J = 13 Hz), 3.03 (1H, brd, J = 13 Hz), 2.94 (1H, brdd, J = 12,3 Hz), 2.77 (1H, ddddd, J = 14,14,14,4,4 Hz), 2.69 (1H, brd, J = 13 Hz), 2.57 (1H, dd, J = 17,6 Hz), 2.33 (1H, dd, J = 13,13 Hz), 2.27 (1H, brd, J = 17 Hz), 2.06 (1H, brdd, J = 13.5,4.5 Hz), 1.50 (1H, m), 1.37 (1H, ddd, J = 12,12,3 Hz), 0.91 (3H, d, J = 6 Hz); ^{13}C nmr (100.6 MHz): 208.4 (s), 74.8 (s), 63.8 (t), 59.6 (t), 48.6 (d), 41.9 (t), 41.2 (t), 37.3 (d), 36.1 (d), 34.5 (t), 25.4 (d), 23.4 (t), 22.6 (q), 21.4 (t), 19.8 (t), 17.4 (t); hreims: m/z calcd. for $C_{16}H_{25}NO$: 247.1938 found: 247. 1930 (11, M^+) 216 ($C_{15}H_{22}NO$, 3, M^+-CH_3), 190 ($C_{12}H_{16}NO$, 100, M^+-C_4H_9), 162 ($C_{11}H_{16}N$, 6, M^+-C_4H_9,-CO), 134 ($C_9H_{12}N$, 9, M^+-C_4H_9,-CO,-C_2H_4).

B3.6. Acetylannofoline, L17 (47).

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* Dry organic perchlorates are explosive and therefore must be handled with extreme precaution.

** The gc / ms and hreims of base **53** indicate the presence of a minor component of molecular formula $C_{16}H_{23}NO$ (245).

The sample of base L17 obtained from Marion and Manske's samples contains a small amount of lycopodine (**6a**). Lycopodine was separated by pipette column chromatography (alumina, dichloromethane, 1% methanol in dichloromethane) to afford pure L17, tlc: Rf 0.23 (1% methanol in dichloromethane); $[\alpha]_D = -84.0^{o}$ ($\underline{c} = 0.25$, methanol, perchlorate); ftir $\upsilon_{max}\,cm^{-1}$: 1739, 1712, 1232; ^1H nmr (400 MHz): 4.84 (1H, app.q, J = 3 Hz, H-5eq), 3.24 (1H, ddd, J = 14,14,3 Hz, H-1ax), 3.16 (1H, ddd, J = 12,12,3 Hz, H-9ax), 2.81 (1H, ddq, J = 10,10,6 Hz, H-15ax), 2.65 (1H, dm, J = 14 Hz, H-1eq), 2.58 (1H, dm, J = 12 Hz, H-9eq), 2.34 (1H, dt, J = 12.5,3 Hz, H-4ax), 2.26 (1H, ddd, J = 5,3,3 Hz, H-7eq), 2.23 (1H, dd, J = 14,10 Hz, H-14ax), 2.09 (1H, dt, J = 15,2 Hz, H-6eq), 1.92 (3H, s, $C\underline{H}_3CO_2$-), 1.12 (3H, d, J = 6 Hz, H-16); ^{13}C nmr: see table 20; hreims: m/z calcd. for $C_{18}H_{27}NO_3$: 305.1992, found 305.1983 (3, M^+), 246 ($C_{16}H_{24}NO$, 6, M^+-CH_3CO_2), 234 ($C_{14}H_{20}NO_2$, 100, M^+-C_4H_7O), 174 ($C_{12}H_{16}N$, 78, M^+-C_4H_7O,-CH_3CO_2H), 146 ($C_{10}H_{12}N$, 23, M^+-C_4H_7O,-CH_3CO_2H,-C_2H_4).

Oxidation of α-lofoline (45) to dehydro-α-lofoline (46).

α-Lofoline (**45**) (30 mg) dissolved in dichloromethane (2 mL) was added to a suspension of pyridinium chlorochromate (51 mg) in dichloromethane (3 mL) at 0°C. The mixture was stirred at 0°C for 30 minutes, warmed to room temperature, and stirred at room temperature for 6 hrs. The reaction mixture was filtered through a pipette column over alumina (chloroform, 1% methanol in chloroform). The chloroform fraction was concentrated in *vacuo* to afford **46** (23 mg, 77%) and the methanol-chloroform fraction afforded the starting alcohol (4.2 mg). Dehydro-α-lofoline (**46**) was converted to its perchlorate (60% $HClO_4$), which crystallizes from methanol / ethyl acetate as colourless prisms, mp: 248.5-249.5°C; tlc: Rf 0.30 (1% methanol in dichloromethane); $[\alpha]_D = -53^{o}$ ($\underline{c} = 0.31$, methanol, perchlorate); ftir $\upsilon_{max}\,cm^{-1}$: 1739, 1707, 1235; ^1H nmr (400 MHz):

4.94 (1H, app.q, J = 3 Hz, H-5eq), 3.29 (1H, ddd, J = 14,14,3 Hz, H-1ax) 3.16 (1H, ddd, J = 13,13,2 Hz, H-9ax), 3.07 (1H, dd, J = 14,10 Hz, H-14ax), 2.82 (1H, ddq, J = 10,5,7 Hz, H-15ax), 2.60 (2H, m, H-1eq,H-9eq), 2.41 (1H, ddd, J = 12,3,3 Hz, H-4ax), 2.32 (1H, brs $W_{1/2}$ = 10 Hz, H-7eq), 1.94 (3H, s, $\underline{CH_3}CO_2$-); 1.30 (3H, d, J = 7 Hz, H-16); ^{13}C nmr: see table 20; hreims: m/z calcd. for $C_{18}H_{27}NO_3$: 305.1992, found: 305.1986 (7, M^+), 246 ($C_{16}H_{24}NO$, 5, M^+-CH_3CO_2), 234 ($C_{14}H_{20}NO_2$, 100, M^+-C_4H_7O), 174 ($C_{12}H_{16}N$, 61, M^+-C_4H_7O,-CH_3CO_2H), 146 ($C_{10}H_{12}N$, 20 M^+-C_4H_7O,-CH_3CO_2H,-C_2H_4).

Preparation of acetylannofoline (47) from dehydro-α-lofoline (46).

NaOH pellets (0.187 g) dissolved in ethanol (98%):water; 9:1 (10 mL) was added to dehydro-α-lofoline (**46**) (12 mg) and the mixture was heated under reflux for 2 hrs. Most of the ethanol was evaporated in *vacuo*, the residue was diluted with water, and the aqueous solution was extracted several times with chloroform. The chloroform extract was concentrated in *vacuo* and purified by filtration through a pipette column over alumina (Skellysolve B, chloroform) to afford annofoline (51) in quantitative yield (by ^{1}H nmr). Without further purification **51** was heated under reflux in Ac_2O (8 mL) for 1hr, the mixture was cooled to room temperature, and the excess Ac_2O was decomposed by dropwise addition of water. The mixture was made basic (10% NaOH) and extracted several times with chloroform. The chloroform extract was concentrated in *vacuo* and purified by filtration through a pipette column over alumina (1% methanol in chloroform) to afford acetylannofoline (47) (11 mg, 92%). Acetylannofoline was purified by conversion to its perchlorate (60% $HClO_4$) and recrystallized from methanol / acetone as colourless needles, mp: 268°C (starts to discolour) 280-283°C (liquifies) (292°C lit[11]).

Preparation of dihydro-O-acetylacrifoline (48).

O-acetylacrifoline (**52**) (13 mg), methanol (10 mL), and PtO_2 (3 mg) in a hydrogenation bottle were subjected to hydrogen at 40 psi for 2 hrs. The catalyst was filtered off and the methanol was concentrated in *vacuo* to afford crude product in quantitative yield. The crude reaction product afforded dihydro-O-acetylacrifoline (**48**), (10.7 mg, 82%) and acetylannofoline (**47**), (0.5 mg, 4%) after chromatography over alumina (0.6% methanol in dichloromethane). Dihydro-O-acetylacrifoline was converted to its perchlorate, which crystallizes from methanol / ethyl acetate as colourless prisms, mp: 237-237.5°C; tlc: Rf 0.41 (1% methanol in dichloromethane); $[\alpha]_D = -56^o(\underline{c} = 0.12$, methanol); ftir υ_{max} cm^{-1}: 2951, 2924, 2866, 2854, 2805 (*Bohlmann bands*), 1734, 1697, 1238, 1229; ^1H nmr (400 MHz): 4.97 (1H, ddd, J = 4,3,3 Hz, H-5eq), 2.60 (1H, ddq, J = 10,10,6.5 Hz, H-15ax), 2.46 (2H, m), 2.37 (2H, m), 2.29 (1H, m, H-7eq), 2.19 (1H, ddd, J = 14.5,3,3 Hz, H-6eq), 2.11 (1H, dd, J = 14.5,10 Hz, H-14ax), 1.94 (3H, s, $C\underline{H}_3CO_2$-), 1.89 (1H, ddd, J =14.5,5,4 Hz, H-6ax), 1.78-1.42 (10H, m), 1.36 (1H, brdm, J = 13 Hz), 1.17 (3H, d, J = 6.5 Hz, H-16); ^{13}C nmr: see table 20; hreims: m/z calcd. for $C_{18}H_{27}NO_3$: 305.1992, found: 305.1987 (23, M^+), 246 ($C_{16}H_{24}NO$, 15, M^+-CH_3CO_2), 234 ($C_{14}H_{20}NO_2$, 100, M^+-C_4H_7O), 174 ($C_{12}H_{16}N$, 24, M^+-C_4H_7O,-CH_3CO_2H), 146 ($C_{16}H_{12}N$, 9, M^+-C_4H_7O,-CH_3CO_2H,-C_2H_4).

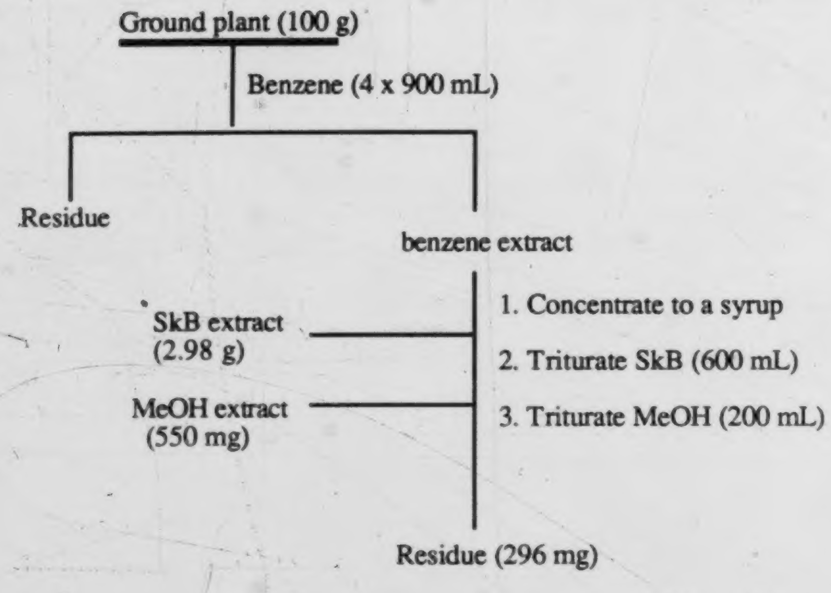
C Mentha arvensis.

C1. Plant collection and extraction.

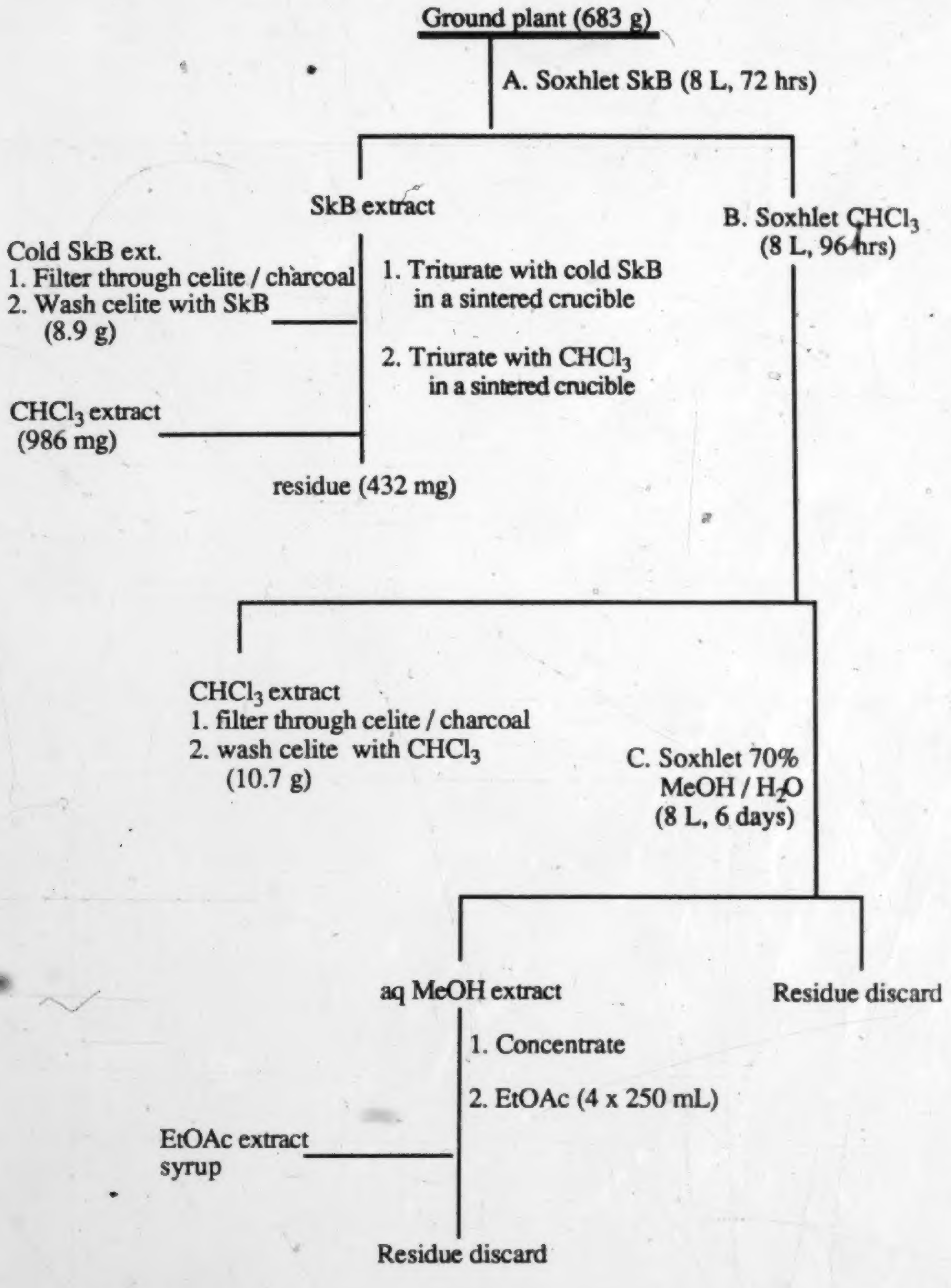
Mentha arvensis was collected at the Devonian Botanical Gardens, Devon, Alberta, during early September 1982. Whole plant was air dried for several weeks and ground. The ground material was extracted using two different procedures as described below.

In a first extraction, *M. arvensis* was extracted using a procedure developed by Inamdar and coworkers for assay of forskolin in *Coleus forskhlii*[15] (scheme 25). The ground plant was suspended in benzene at *ca* 40°C in an Erlenmeyer flask. The suspension was constantly stirred by means of a mechanical stirrer for 6 hrs. The extraction with benzene was repeated 3 more times. The benzene solution was concentrated to a syrup in *vacuo* and the syrup was triturated in a sintered crucible successively with Skellysolve B and methanol. Each of the Skellysolve B and methanol extracts was concentrated under reduced pressure and then analysed for the presence of forskolin by tlc. The thin layer chromatograms of the crude extracts were compared with those of an authentic sample of forskolin using a variety of solvent systems for development.

In a second extraction, ground plant was extracted successively with Skellysolve B, chloroform, and 70% methanol in water in a Soxhlet apparatus (scheme 26). Most of the methanol was concentrated under reduced pressure and extracted 4 times with ethyl acetate. Chlorophylls in each of the Skellysolve B and chloroform extracts were removed by filtration through charcoal:celite; *ca* 1:1 and washing with Skellysolve B and chloroform, respectively.


Ground plant (100 g)
Benzene (4 x 900 mL)
Residue
benzene extract
SkB extract (2.98 g)
MeOH extract (550 mg)
1. Concentrate to a syrup
2. Triturate SkB (600 mL)
3. Triturate MeOH (200 mL)
Residue (296 mg)

Scheme 25. Extraction of *M. arvensis* by percolation with benzene.


Ground plant (683 g)
A. Soxhlet SkB (8 L, 72 hrs)
SkB extract
B. Soxhlet CHCl3 (8 L, 96 hrs)
Cold SkB ext.
1. Filter through celite / charcoal
2. Wash celite with SkB
(8.9 g)
1. Triturate with cold SkB in a sintered crucible
2. Triurate with CHCl3 in a sintered crucible
CHCl3 extract (986 mg)
residue (432 mg)
CHCl3 extract
1. filter through celite / charcoal
2. wash celite with CHCl3
(10.7 g)
C. Soxhlet 70% MeOH / H2O (8 L, 6 days)
aq MeOH extract
Residue discard
1. Concentrate
2. EtOAc (4 x 250 mL)
EtOAc extract syrup
Residue discard

Scheme 26. Extraction of *M. arvensis* in a Soxhlet apparatus.

C2. Isolation.

The crude *M. arvensis* extracts were fractionated by flash column chromatography. The chromatography fractions were monitored by tlc using glass or aluminium silica gel precoated commercial plates. Unless otherwise noted, Rf values refer to silica gel plates using Skellysolve B:ethyl acetate:acetic acid; 75:25:1 as developing solvent.

C2.1. Isolation of 2,4,6,7,7a-Hexahydro-7a-acetyl-3,6-dimethyl benzofuran-2-one (54).

Concentration of the Skellysolve B extract precipitates colourless plates, which were crystallized from Skellysolve B / ethyl acetate to afford lactone **54** as colourless needles, mp: 190-191°C (188°C, lit[43]); tlc: Rf 0.10; $[\alpha]_D = -61^\circ$ ($\underline{c}$ = 0.15, methanol) (-61.6°, lit[43]); uv ($\underline{c}$ = 0.011, methanol) λ_{max} nm ($\log \varepsilon$): 215 (4.39), 260 (3.7) (methanol- NaOH); ftir υ_{max} cm^{-1}: 3345, 1748, 1693, 1119; ^{1}H nmr (400 MHz): 3.03 (1H, brs, O$\underline{H}$), 2.68 (1H, ddd, J = 14,4,2 Hz, H-4eq), 2.36 (2H, m, H-4ax,H-7eq), 1.98 (2H, m, H-5eq,H-6eq), 1.82 (3H, d, J = 2 Hz, H-8), 1.27 (1H, ddd, J = 13,13,1.5 Hz, H-7ax), 1.02 (1H, brdddd, J = 13,13,13, 4 Hz, H-5ax), 0.98 (3H, d, J = 7 Hz, H-9); ^{13}C nmr (100.6 MHz): 174.6 (s, C-2), 162.9 (s, C-3a), 121.7 (s, C-3), 105.5 (s, C-7a), 47.1 (t, C-4)*, 36.1 (t, C-7)*, 30.5 (d, C-6), 25.2 (d, C-5), 21.3 (q, C-8), 7.9 (q, C-9); cims: m/z 200 (100, M$^+$ + 18), 382 (6, 2M$^+$ + 18); hreims: m/z calcd. for $C_{10}H_{14}O_3$: 182.0943, found: 182.0946 (10, M$^+$), 164 ($C_{10}H_{12}O_2$, 2); 154 ($C_9H_{14}O_2$, 100), 139 ($C_8H_{11}O_2$, 48), 137 ($C_9H_{13}O$, 31), 109 (C_8H_{13}, 49), 81 (C_6H_9, 41), 67 (C_5H_7, 42), 53 (C_4H_5, 21).

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* Signals may be reversed.

Preparation of 2,4,6,7,7a-Hexahydro-7a-acetyl-3,6-dimethylbenzofuran-2-one (55).

The lactone **54** (8 mg) and Ac_2O (0.8 mL) in pyridine (3 mL) were heated on a water bath for 2 1/2 hrs. The reaction mixture was cooled to room temperature, the solvent was concentrated in *vacuo*, and the residue purified by flash column chromatography (Skellysolve B:ethyl acetate:acetic acid; 75:25:1) to afford the acetate **55** (5 mg) as an oil, tlc: Rf 0.26; ftir υ_{max} cm^{-1}: 1776, 1700, 1218, 1151, 1130, 975; ^1H nmr (400 MHz): 2.75 (1H, ddd, J = 13.5,5,2 Hz, H-4eq), 2.69 (1H, brddd, J = 13.5,3,3 Hz, H-7eq), 2.17 (1H, m, H-4 ax), 2.05 (3H, s, $C\underline{H}_3CO_2$-), 1.97 (1H, m, H-5eq), 1.88 (1H, m, H-6ax), 1.84 (3H, d, J = 2 Hz, H-8), 1.14 (1H, dd, J = 13,13 Hz, H-7ax), 1.02 (1H, brdddd, J = 13,13,13,4 Hz, H-5ax), 0.98 (3H, d, J = 7 Hz, H-9); hreims: m/z calcd. for $C_{12}H_{16}O_4$: 224.1049, found: 224.1049 (8, M^+), 182 ($C_{10}H_4O_3$, 34), 165 ($C_{10}H_{13}O_2$, 91), 164 ($C_{10}H_{12}O_2$, 100), 154 ($C_9H_{14}O_2$, 71), 149 ($C_9H_9O_2$, 20), 139 ($C_8H_{11}O_2$, 38), 137 ($C_9H_{13}O$, 21), 136 ($C_9H_{12}O$, 17), 111 ($C_6H_7O_2$, 21), 109 (C_8H_{13}, 16), 81 (C_6H_9, 28), 67 (C_4H_7, 31), 53 (C_4H_5, 34).

C2.2. Isolation of β-sitosterol (56).

The Skellysolve B extract (4.5 g) was separated by flash column chromatography (25% ethyl acetate in skellysolve B) into four fractions **a** to **d**. β-Sitosterol was obtained from a portion of fraction **b** (482 mg) by preparative tlc (Skellysolve B:ethyl acetate:acetic acid; 75:25:1). β-Sitosterol crystallizes from ethanol / ether as colourless plates, mp: 135-137°C (139°C, lit[47]); tlc: Rf 0.22; ftir υ_{max} cm^{-1}: 3429, *ca* 1668 (weak, C=C), 1060; ^1H nmr (400 MHz): 5.36 (1H, brs, $W_{1/2}$ = 10 Hz, H-6), 3.54 (1H, m, H-3); hreims: m/z calcd. for $C_{29}H_{50}O$: 414.3864, found: 414.3874 (100, M^+), 412 ($C_{29}H_{48}O$, 20), 399 ($C_{28}H_{47}O$, 25), 396 ($C_{29}H_{48}$, 37), 381 ($C_{28}H_{45}$, 21), 329 ($C_{24}H_{41}$, 29), 303

($C_{22}H_{39}$, 34), 273 ($C_{19}H_{29}O$, 20), 255 ($C_{19}H_{27}$, 28), 213 ($C_{16}H_{21}$, 25), 119 (C_9H_{11}, 22).

C2.3. Isolation of mixture A of oleanolic (57) and ursolic acid (58)

A portion of the chloroform extract (841 mg) was separated by flash column chromatography (2% methanol in chloroform) to afford a major component, as a white gum (324 mg). The white gum was triturated with dichloromethane and the dichloromethane extract was concentrated in *vacuo* to afford an off white mixture A (200 mg) containing the triterpenes **57** and **58**.

Preparation of 3β-acetyl-12α-bromooleanan-28-13β-olide (61) and 3β-acetylursen-28-13β-olide (63).

A portion of the mixture A above (70 mg) and Ac_2O (0.6 mL) in pyridine (6 mL) were stirred at room temperature overnight. The reaction mixture was concentrated in *vacuo* and the residue was separated by flash column chromatography (Skellysolve B:ethyl acetate:acetic acid; 75:25:1) to afford a mixture of 3β-acetyloleanolic (**59**) and 3β-acetylursolic acid (**60**) (55 mg). Bromine solution (6 mL) was added to the acetate mixture (40 mg) dissolved in methanol (5 mL). The reaction mixture was allowed to stand at room temperature for 30 minutes, concentrated in *vacuo*, and the residue separated by flash column chromatography (ethyl acetate in Skellysolve B, 3%, 6%, 10%) to afford the lactones **61** (9 mg), **62** (5 mg), and recovered mixture (24 mg) containing **59**, **60**, and other unidentified reaction product(s).

Lactone 61

Lactone **61** crystallizes from Skellysolve B / dichloromethane as colourless needles, mp: 244-246°C (215-216°C, lit[51b]); tlc: Rf 0.50; ftir υ_{max} cm^{-1}: 1774, 1734, 1224 (1770, 1725, 1240, 1180, 1160 lit[51b]), ^{1}H nmr (400 MHz): 4.54 (1H, brdd, J = 10,6 Hz, H-3α), 4.28 (1H, brdd, J = 4,1.5 Hz, H-12), 2.06 (3H, s, $C\underline{H}_3CO_2$-), 1.45, 1.30, 1.01, 0.92, 0.90, 0.88, 0.87 (each 3H, s,); cims: m/z 596 (99, (M$^+$ +2) + 18), 595 (36, (M$^+$ + 1) + 18), 594 (100, (M$^+$) + 18); hreims: m/z calcd. for $C_{32}H_{49}O_4Br$: 578.2796, 497 (C_{32},$H_{49}O_4$, 5), 469 ($C_{31}H_{49}O_3$, 6,), 451 ($C_{31}H_{47}O_2$, 10), 437 ($C_{30}H_{45}O$, 79), 391 ($C_{29}H_{43}$, 9), 249 ($C_{16}H_{25}O_2$, 35), 189 ($C_{14}H_{21}$, 100).

Lactone 63.

mp: 255-260°C, tlc: Rf 0.43; ftir υ_{max} cm^{-1}: 1755, 1734, 1246; ^{1}H nmr (400 MHz): 5.94 (1H, dd, J = 10,1 Hz, H-12); 5.56 (1H, dd, J = 10,2.5 Hz, H-11), 4.52 (1H, dd, J = 11,5 Hz, H-3α), 2.08 (3H, s, $C\underline{H}_3CO_2$-), 2.14 (1H, ddd, 7 J = 13,13, 6 Hz), 1.98 (1H, brs, $W_{1/2}$ = 6 Hz), 1.16 (3H, s), 1.06 (3H, s), 1.00 (3H, d, J = 6 Hz), 0.94 (3H, perturbed d, J = 6 Hz), 0.94 (3H, s), 0.86 (3H, s), ^{13}C nmr (50.3 MHz): 179.8 (s), 170.9 (s), 133.2 (d), 128.9 (d), 89.6 (s), 80.6 (d), 60.6 (d), 54.9 (d), 53.0 (d), 45.1 (t), 42.0 (t), 40.3 (d), 38.0 (t), 37.9 (t), 36.3 (t), 31.4 (t), 31.2 (t), 30.8 (t), 27.7 (q), 25.6 (t), 23.3 (t), 22.8 (t), 21.2 (q), 19.1 (q), 18.9 (q), 18.0 (q), 17.8 (q), 17.7 (t), 17.6 (t), 16.1 (q), 16.0 (q); cims: m/z 514 (100, M$^+$+18), 497 (63, M$^+$+1) hreims: m/z calcd. for $C_{32}H_{48}O_4$: 496.3554, found: 496.3557 (100, M$^+$), 468 ($C_{31}H_{48}O_3$, 5), 452 ($C_{31}H_{48}O_2$, 43), 451 ($C_{31}H_{47}O_2$, 34), 436 ($C_{30}H_{44}O_2$, 26), 285 ($C_{19}H_{25}$, 21), 233 ($C_{15}H_{21}O_2$, 57), 201 ($C_{15}H_{21}$, 23), 189 ($C_{14}H_{21}$, 40), 187 ($C_{14}H_{19}$, 23), 145 ($C_{11}H_{13}$, 23), 133 ($C_{10}H_{13}$, 20), 119 (C_9H_{11}, 38), 107 (C_8H_{11}, 26).

Preparation of methyl 3β-acetylursolate (64).

Excess ethereal diazomethane was added to a mixture of 3β-acetyloleanolic (59) and 3β-acetylursolic acid (60) (41 mg) dissolved in minimum ether. The mixture was stirred at room temperature for 5-10 minutes, the reaction mixture was concentrated in *vacuo*, and purified by flash column chromatography (Skellysolve B:ethyl acetate; 9:1) to afford a mixture of methyl ester acetate in quantitative yield. Repeated fractional crystallization from methanol afforded pure ester acetate **64**, mp: 226-229°C; tlc: Rf 0.54; ftir υ_{max} cm^{-1}: 1733, 1244; ^{1}H nmr: 5.25 (1H, t, J = 4 Hz, H-12), 4.50 (1H, brdd, J = 10,5 Hz, H-3α), 3.60 (3H, s, $-CO_2C\underline{H}_3$), 2.23 (1H, d, J = 11 Hz, H-18β), 2.05 (3H, s, $C\underline{H}3CO_2$-), 2.00 (1H, ddd, J = 15,15,5 Hz), 1.91 (2H, dd, J = 9,4 Hz), 1.08 (3H, s), 0.95 (3H, s), 0.95 (3H, perturbed d, J = 6 Hz), 0.87 (3H, s), 0.87 (3H, d, J = 7 Hz), 0.86 (3H, s), 0.74 (3H, s); hreims: m/z calcd. for $C_{33}H_{52}O_4$: 512.3868, found: 512. 3870 (4, M$^+$), 452 ($C_{31}H_{48}O_2$, 7), 262 ($C_{17}H_{26}O_2$, 100), 249 ($C_{16}H_{25}O_2$, 21), 203 ($C_{15}H_{23}$, 79), 202 ($C_{15}H_{22}$, 13), 189 ($C_{14}H_{21}$, 25), 133 ($C_{10}H_{13}$, 43).

Preparation of 3β-acetyloleanolic acid (59).

Excess zinc dust was added in several portions over a period of 5 hrs to the bromolactone 61 (8.2 mg) dissolved in ether (2 mL) / acetic acid (5 drops). The suspension was stirred at room temperature and the excess zinc dust was destroyed by dropwise addition of water. The mixture was diluted with ether and the ether solution was decanted over celite. The ether solution was dried (anhydrous Na_2SO_4) and concentrated under reduced pressure to afford the acetate **59** (4.3 mg, 86%). Acetyloleanolic acid (59) was recrystallized from Skellysolve B-dichloromethane, mp: 250-253°C; tlc: Rf 0.50; $[\alpha]_D = +72^o$ ($\underline{c}$ = 0.2, chloroform); ftir υ_{max} cm^{-1}: 1735, 1695, 1244; ^{1}H nmr (400 MHz): 5.26 (1H, brt, J = 3.5 Hz, H-12), 4.44 (1H, brdd, J = 9,7

Hz, H-3α), 2.78 (1H, brdd, J = 14,4.5 Hz, H-18β), 2.05 (3H, s, C$\underline{H}_3CO_2$-), 1.99 (1H, brddd, J = 14,14,5 Hz), 1.14, 0.93 ,0.94, 0.92, 0.88, 0.86, 0.77 (each 3H, s); cims: m/z 516 (100, M^++18), hreims: m/z calcd. for $C_{32}H_{50}O_4$: 498.3711, found: 498.3712 (1, M^+), 452 ($C_{31}H_{48}O_2$, 2), 438 ($C_{30}H_{46}O_2$, 4), 423 ($C_{29}H_{43}O_2$, 2), 248 ($C_{16}H_{25}O_2$, 100), 203 ($C_{15}H_{23}$, 69), 190 ($C_{14}H_{22}$, 23).

C2.4. Isolation of methyl 3β,19α-dihydroxy-urs-12-en-28-oate (65).

The chloroform extract (796 mg) dissolved in minimum ether containing a few drops of methanol was treated with excess ethereal diazomethane at room temperature for 5-10 minutes. The reaction mixture was concentrated in *vacuo* and the residue was separated by flash column chromatography (3%, 15%, ethyl acetate in Skellysolve B, 25% ethyl acetate in chloroform) to afford methyl palmitate (41.2 mg), an unidentified methyl ester ($C_{20}H_{34}O_3$, 14.4 mg), a mixture of methyl oleanolate and ursolate (425 mg), an oil containing methyl pomolate (65) (26 mg), and lactone (54) (197 mg). The oil containing methyl pomolate was crystallized from dichloromethane / Skellysolve B to afford crystalline methyl pomolate (9 mg), mp: 79-80°C (105-115°C, pet ether lit^{55}); tlc: Rf 0.10; ftir υ_{max} cm^{-1}: 3610, 3540, 3420 (OH), 1718, 1456, 1207, 1153, 909, 734; ^{1}H nmr (400 MHz): 5.36 (1H, t, J = 3 Hz, H-12); 3.60 (3H, s, -$CO_2C\underline{H}_3$), 3.22 (1H, dd, J = 11,5 Hz, H-3ax), 2.60 (1H, brs, $W_{1/2}$ = 4 Hz, H-18β), 2.50 (1H, ddd, J = 13,13,5 Hz), 1.99-1.94 (2H, m, 2H-11), 1.25 (3H, s, H-29), 1.21 (3H, s, H-27), 0.99 (3H, s), 0.94 (3H, d, J = 7 Hz, H-30), 0.90 (3H, s), 0.78 (3H, s), 0.70 (3H, s); cims: m/z 504 (8, M^++1); hreims: m/z calcd. for $C_{31}H_{50}O_4$: 486.3711, found: 486.3701 (12, M^+), 468 ($C_{31}H_{48}O_3$, 15), 426 ($C_{29}H_{46}O_2$, 29), 278 ($C_{17}H_{26}O_3$, 9), 260 ($C_{17}H_{24}O_2$, 17), 220 ($C_{15}H_{24}O$, 21), 207 ($C_{14}H_{23}O$, 32), 201 ($C_{15}H_{21}$, 31), 190 ($C_{14}H_{22}$, 35), 179 ($C_{11}H_{15}O_2$, 100), 146 ($C_{11}H_{14}$, 47), 99 ($C_6H_{11}O$, 12).

Acetylation of 65.

The mother liquors (11.3 mg) obtained from crystallization of **65** above and Ac_2O (10 drops) in pyridine (2 mL) were stirred at room temperature overnight. The reaction mixture was concentrated in *vacuo* and the residue was purified by flash column chromatography (3%, 10% ethyl acetate in Skellysolve B) to afford the acetate **66** (7.1 mg), which was crystallized from dichloromethane-Skellysolve B, mp: 236-237°C (235-238°C lit[55]); tlc: Rf 0.35; ftir υ_{max} cm^{-1}: 3530, 1723, 1246; ^1H nmr (400 MHz): 5.36 (1H, t, J = 3 Hz, H-12), 4.50 (1H, brdd, J = 9,7 Hz, H-3β), 3.60(3H, s, $-CO_2C\underline{H}_3$), 2.60 (1H, brs, $W_{1/2}$ = 4 Hz, H-18β), 2.50 (1H, ddd, J = 14,14,5 Hz), 2.05 (3H, s, $C\underline{H}_3CO_2$-), 2.00-1.94 (2H, m, 2H-11), 1.25 (3H, s, H-29β), 1.22 (3H, s, H-27), 0.97 (1H, s, $-O\underline{H}$), 0.95 (3H, d, J = 7 Hz, H-30α), 0.93 (3H, s), 0.87 (3H, s), 0.86 (3H, s), 0.68 (3H, s); hreims: m/z calcd. for $C_{33}H_{52}O_5$: 528.3817, found: 528.3814 (2, M^+), 510 ($C_{33}H_{50}O_4$, 2), 469 ($C_{31}H_{49}O_3$, 14), 468 ($C_{31}H_{48}O_3$, 39), 260 ($C_{17}H_{24}O_2$, 77), 249 ($C_{16}H_{25}O_2$, 5), 201 ($C_{15}H_{21}$, 29), 219 ($C_{15}H_{23}O$, 25), 179 ($C_{11}H_{15}O_2$, 100), 146 ($C_{11}H_{14}$, 60).

Oxidation of 65.

A fraction containing **65** (5 mg) dissolved in dichloromethane (1 mL) was added to a suspension of pyridinium chlorochromate (8.6 mg) in dichloromethane (1 mL) at 0°C. The mixture was stirred at 0°C for 30 minutes, warmed to room temperature, and stirred at room temperature for 5 hrs. The reaction mixture was filtered through florosil (dichloromethane), the dichloromethane extract concentrated in *vacuo*, and the residue purified by flash column chromatography (10% ethyl acetate in Skellysolve B) to afford the ketone **67** (3 mg), tlc: Rf 0.25; ftir υ_{max} cm^{-1}: 3530, 1721, 1704, 1457, 1167, 1151, 732; ^1H nmr (400 MHz): 5.38 (1H, t, J = 4 Hz, H-12), 2.62 (1H, brs, $W_{1/2}$ = 4 Hz, H-

18β), 2.51-2.50 (2H, m), 2.36 (1H, ddd, J = 16,6.5,3.5 Hz), 2.03 (2H, dd, J = 9,4 Hz, 2H-11), 1.81 (1H, ddd, J = 13,7,3.5 Hz), 1.27 (3H, s, H-29β), 1.22 (3H, s, H-27), 1.09 (3H, s), 1.06 (3H, s), 1.05 (3H, s), 0.95 (3H, d, J = 7 Hz, H-30α), 0.75 (3H, s); cims: m/z 502 (100, M^{+}+18); hreims: m/z calcd. for $C_{31}H_{48}O_4$: 484.3554, found: 484.3551 (10, M^{+}), 466 ($C_{31}H_{46}O_3$, 17), 424 ($C_{29}H_{44}O_2$, 61), 407 ($C_{29}H_{43}O$, 11), 352 ($C_{25}H_{36}O$, 34), 247 ($C_{16}H_{23}O_2$, 48), 219 ($C_{15}H_{23}O$, 25), 218 ($C_{15}H_{22}O$, 29), 205 ($C_{14}H_{21}O$, 48), 201 ($C_{15}H_{21}$, 54), 200 ($C_{15}H_{20}$, 23), 187 ($C_{14}H_{19}$,49), 179 ($C_{11}H_{15}O_2$, 100), 147 ($C_{11}H_{15}$, 34), 146 ($C_{11}H_{14}$, 86), 145 ($C_{11}H_{13}$, 28), 144 ($C_{11}H_{12}$, 33), 99 ($C_6H_{11}O$, 27).

D. *Geranium viscosissimum* and *Eriogonum umbellatum*.

D1. Plant collection and extraction.

Geranium viscosissimum was collected in Alberta, at Beaver Mines, along the road side of Castle river during early July 1985. *Eriogonum umbellatum* was collected in Alberta along highway 12, 20 km north of Rimbey during late August 1985. Specimens* of both plants are deposited in the herbarium at the Devonian Botanic Gardens, Devon, Alberta.

Whole plant of each of *G. viscosissimum* and *E. umbellatum* was air dried for several weeks, ground, and extracted with 60% methanol in water for 24 hrs using a Soxhlet apparatus as shown in scheme 27. Most of the methanol was evaporated under reduced pressure at 40°C. The aqueous residue was treated to successive liquid-liquid extraction with Skellysolve B, ether, and ethyl acetate. The aqueous residue was basified to pH *ca* 9 ($NaHCO_3$) and extracted with ethyl acetate to afford crude bases. The crude extracts from *G. viscosissimum* (200 g) and *E. umbellatum* (150 g) are shown in table 29.

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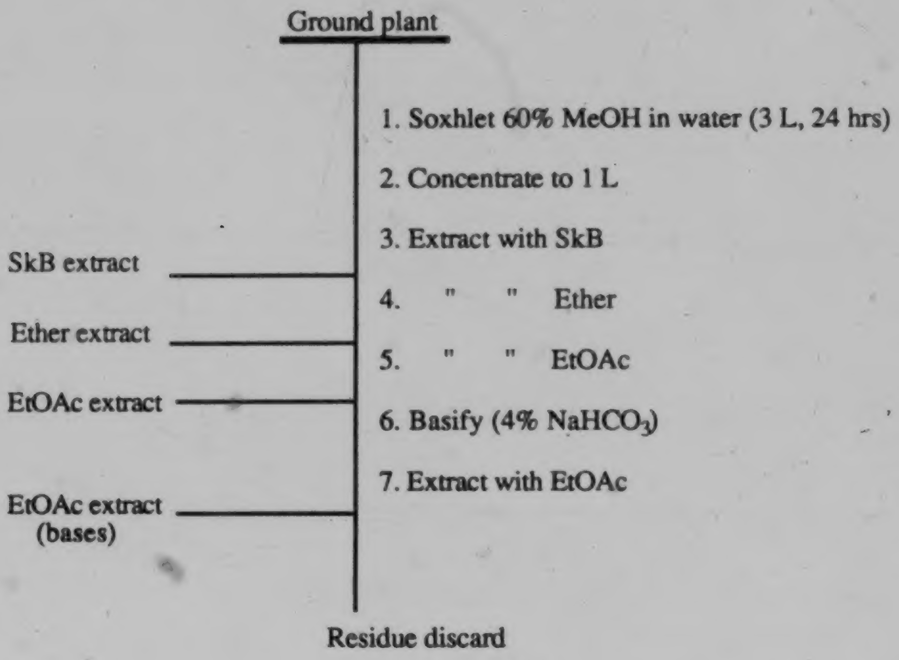
* Accession No's: 2105 (*E. umbellatum)*, 2106 (G. viscosissimum).


Ground plant
1. Soxhlet 60% MeOH in water (3 L, 24 hrs)
2. Concentrate to 1 L
3. Extract with SkB
4. " " Ether
5. " " EtOAc
6. Basify (4% NaHCO3)
7. Extract with EtOAc
SkB extract
Ether extract
EtOAc extract
EtOAc extract (bases)
Residue discard

Scheme 27. Extraction of *G. viscosissimum* and *E. umbellatum* in a Soxhlet apparatus.

Table 29 Crude extracts of *G. viscosissimum* and *E. umbellatum*.

	Extract	Quantity (g)	Observation
	SkB	1.6	Δ
G. viscosissimum	Ether	0.86	68 69 71
	EtOAc	4.09	Δ
	EtOAc (bases)	0.25	69 71
	SkB	1.93	Δ
E. umbellatum	Ether	2.07	56 69 72
	EtOAc	6.90	69 71 72 74 77 79 81 83 85 87
	EtOAc (bases)	0.089	69 77 79

Δ, Extract not investigated chemically.

D2. Biological assay of crude extracts and pure metabolites.

The crude plant extracts and pure metabolites were screened for antibacterial activities using the modified Kirby-Bauer test. In this test, an inoculum of the microorganism to be tested is prepared from commercial Bactrol disks (Difco) in the following way: a disk containing the microorganism is aseptically placed in 2 mL of Mueller-Hinton broth previously sterilized and cooled. The mixture is incubated at 35-37°C for 3 hrs. The inoculum is lightly brushed onto the surface of a Mueller-Hinton agar plate (6 mm in depth) with a cotton swab. The test solution is prepared in a suitable solvent at concentrations of 5% / mL (crude extract) and 2% / mL (pure compound). With tweezers, a paper disk (6 mm in diameter) is soaked with the test solution and air dried. The paper disk is firmly placed onto the surface of the agar plate and the agar plate is incubated at 37°C for 24 hrs. The bioassay results with the extracts of *G. viscosissimum* and *E. umbellatum* are shown in table 24.

D3. Isolation.

The metabolites were separated by flash column chromatography or by gel filtration chromatography on Sephadex LH20 or G10 using methanol or methanol / water mixtures as eluants or by column chromatography on cellulose (Brickmann avicel microcrystalline cellulose particle size: 38μ). The chromatography fractions were monitored by tlc using silica gel plates.

D3.1. Isolation of compounds 68, 69 and 71.

The ether extract of *G. viscosissimum* (642 mg) was separated by flash column chromatography (benzene:ethyl acetate:acetic acid; 80:20:1, 75:25:1, and pure ethyl acetate) into 8 fractions **a** to **h**. The fractions **a**, **e**, and **f** were purified further.

Salicylic acid (68).

Salicylic acid (**68**) (6.3 mg) was obtained from fraction **a** (7 mg) by pipette column chromatography over silica gel (Skellysolve B:ethyl acetate:acetic acid; 75:25:1). Salicylic acid crystallizes from Skellysolve B / dichloromethane as colourless needles, mp: 134-136°C (134-137°C, authentic sample); tlc: Rf 0.20 (Skellysolve B:ethyl acetate:acetic acid; 75:25:1; ftir υ_{max} cm^{-1}: 3400-2500 (br, OH), 1662, 1612; ^{1}H nmr (400 MHz): 10.4 (1H, brs), 7.94 (1H, dd, J = 2 Hz), 7.54 (1H, ddd, J = 9,9,2 Hz), 7.02 (1H, dd, J = 9,2 Hz), 6.95 (1H, ddd, J = 9,9,2 Hz); cims: m/z 156 (7, M$^+$+18); hreims: m/z calcd. for $C_7H_6O_3$: 138.0317, found: 138.0321 (59, M$^+$), 120 ($C_7H_4O_2$, 100), 92 (C_6H_4O, 67).

Methyl gallate (69).

Methyl gallate (**69**) (69 mg) was obtained from fraction **e** (96.4 mg) by flash column chromatography (methanol:chloroform:acetic acid; 5:94:1), mp: 157^{o}C; tlc: Rf 0.51 (methanol:chloroform:acetic acid; 4:20:1); ftir υ_{max} cm^{-1} (acetone cast): 3100-3500 (br OH), 1693, 1619, 1440, 1253, 1199, 1004; cims: m/z 202 (100, M$^+$+18); hreims: m/z calcd. for $C_8H_8O_5$: 184.0371, found: 184.0372 (68, M$^+$), 153 ($C_7H_5O_4$, 100), 125 ($C_6H_5O_3$, 20), ^{1}H nmr (acetone-d$_6$, 400 MHz): 7.09 (2H, s), 3.77 (3H, s, -$CO_2C\underline{H}_3$).

Acetylation of methyl gallate (69).

Excess Ac_2O was added to a solution of methyl gallate (**69**) (53 mg) in pyridine (1 mL). The reaction mixture was stirred at room temperature for 2 hrs, concentrated in *vacuo*, and separated by flash column chromatography (Skellysolve B:ethyl acetate:acetic acid; 75:25:1) to afford quantitatively the triacetate **70**. Compound **70** crystallizes from methanol as colourless plates, mp: 121-122^{o}C (120-122^{o}C^{77}); tlc: Rf 0.16 (Skellysolve B:ethyl acetate:acetic acid; 75:25:1); ftir υ_{max} cm^{-1}: 1781, 1726, 1329, 1201, 1154, 1054; hreims: m/z calcd. for $C_{14}H_{14}O_8$: 310.0688, found: 310.0687 (4, M$^+$), 279 ($C_{13}H_{11}O_7$, 2), 268 ($C_{12}H_{11}O_7$, 10), 226 ($C_{10}H_{10}O_6$, 47), 184 ($C_8H_8O_5$, 100); ^{1}H nmr (200 MHz): 7.80 (2H, s), 3.91 (3H, s, -$CO_2C\underline{H}_3$), 2.30 (9H, s, 3 x $C\underline{H}_3CO_2$-).

Gallic acid (71).

Fraction f (45.2 mg) was crystallized from methanol-chloroform to afford the acid **71** as off white needles, mp: 235°C (dec.); tlc: Rf 0.15 (methanol:chloroform:acetic acid; 10:89:1); ^{1}H nmr (acetone-d_6, 200 MHz): 7.12 (2H, s); cims: m/z 188 (100, M^++18); hreims: m/z calcd. for $C_7H_6O_5$: 170.0215, found: 170.0217 (100, M^+), 153 ($C_7H_5O_4$, 77), 125 ($C_6H_5O_3$, 30).

D3.2. Isolation of β-sitosteryl-β-D-glucopyranoside (72).

A portion of the ether extract of *E. umbellatum* (green gum, 950 mg) was separated by flash column chromatography (1% acetic acid in chloroform:ethyl acetate, 4:1, 1:1) to afford six fractions **a** to **f**. The glycoside **72** (7.5 mg) was obtained from fraction f (27 mg) by preparative tlc (ethyl acetate:chloroform:acetic acid, 49:50:1), ^{1}H nmr (acetone-d_6, 200 MHz): 5.35 (1H, brs, H-6), 4.40 (1H, d, J = 8 Hz), 4.37 (1H, dd, J = 14,2 Hz), 4.18 (1H, dd, J = 12,7 Hz), 1.02 (3H, s), 0.97 (3H, d, J = 6 Hz), 0.72 (3H, s); hreims: m/z calcd. for $C_{29}H_{50}O$: 414.3864, found: 414.3876 (2, M^+-sugar portion), 397 ($C_{29}H_{49}$, 66), 381 ($C_{28}H_{45}$, 7), 255 ($C_{19}H_{27}$, 8), 145 ($C_{11}H_{13}$, 9), 119 (C_9H_{11}, 6). Compound **72** was acetylated with excess Ac_2O containing catalytic amount of N,N-dimethylaminopyridine in pyridine (1 mL). The excess Ac_2O was decomposed with water and the reaction mixture was extracted several times with dichloromethane. The dichloromethane extract was washed successively with 5% HCl, 4% $NaHCO_3$, and water, and dried over anhydrous Na_2SO_4. The dichloromethane was concentrated under reduced pressure to afford a white solid, which was purified further by pipette column chromatography over silica gel (chloroform). The chloroform was evaporated in *vacuo*, and the residue was crystallized from dichloromethane / Skellysolve B to afford the tetraacetate derivative **73**, (1.9 mg), mp: 160-161°C; tlc: Rf 0.37 (Skellysolve B:ethyl

acetate:acetic acid; 75:25:1); ftir υ_{max} cm^{-1}: 1746, 1377, 1368, 1262, 1233; ^{1}H nmr (400 MHz): 5.34 (1H, brs, $W_{1/2}$ = 10 Hz, H-6), 5.20 (1H, dd, J = 9.5,9.5 Hz, H-3'), 5.08 (1H, dd, J = 9.5,10 Hz, H-4'), 4.96 (1H, dd, J = 9.5,8 Hz, H-2'), 4.63 (1H, d, J = 8 Hz, H-1'), 4.31 (1H, dd, J = 11.5, 4 Hz, H-6'), 4.17 (1H, dd, J = 11.5,2 Hz, H-6'), 3.65 (1H, dd, J = 10,4,2 H-5'), 3.48 (1H, m, H-3), 2.07 (3H, s, $\mathrm{C\underline{H}_3CO_2}$-), 2.04 (3H, s, $\mathrm{C\underline{H}_3CO_2}$-), 2.02 (3H, s, $\mathrm{C\underline{H}_3CO_2}$-), 2.00 (3H, s, $\mathrm{C\underline{H}_3CO_2}$-), 0.97 (3H, s), 0.91 (3H, d, J = 6 Hz), 0.84 (3H, t, J = 7, Hz), 0.83 (3H, d, J = 7 Hz), 0.81 (3H, d, J = 7 Hz), 0.68 (3H, s); ^{13}Cnmr (100.6 MHz): 170.5 (s), 170.2 (s), 169.3 (s), 169.1 (s), 140.6 (s), 122.1 (d), 99.8, 80.1, 73.2, 71.9 71.88, 69.0, 62.4, 57.0, 56.3, 50.4, 46.1, 42.5, 39.9, 39.1, 37.3, 36.9, 36.2, 34.2, 32.1(2 x C), 29.6, 29.5, 28.3, 26.6, 24.4, 23.3, 21.2, 20.6 (2 x C), 20.5 (2 x C), 19.8, 19.4, 19.2, 18.9, 12.0, 11.9; hreims: m/z calcd. for $C_{29}H_{50}O_{10}$: 414.3876, found, 414.3857 (0.35, M$^+$-sugar portion), 397 ($C_{29}H_{49}$, 56), 396 ($C_{29}H_{48}$, 100), 381 ($C_{28}H_{45}$, 5), 255 ($C_{19}H_{27}$, 6), 145 ($C_{11}H_{13}$, 6), 119 (C_9H_{11}, 4).

D3.3. Isolation of compounds 77 and 79.

The crude bases (87 mg) of *E. umbellatum* were dissolved in ethyl acetate (50 mL) and repartioned into an acid / neutral fraction and a basic fraction as shown in scheme 28. The acid / neutral fraction (65 mg) was separated by flash column chromatography (10% methanol in chloroform containing 1% acetic acid). The major component (35 mg), one spot, by tlc was a mixture of three compounds identified as methyl gallate (**69**), **77**, and **79** (hreims and ^{1}H nmr). A portion of the mixture (22 mg) was acetylated* and separated by flash column chromatography (10% methanol in chloroform) to afford methyl 3,4,5-triacetoxybenzoate (5 mg) and a mixture (2.8 mg) containing the pentaacetates **78** and **80**. The acetates were separated by preparative tlc over silica gel (10% ethyl acetate in chloroform).

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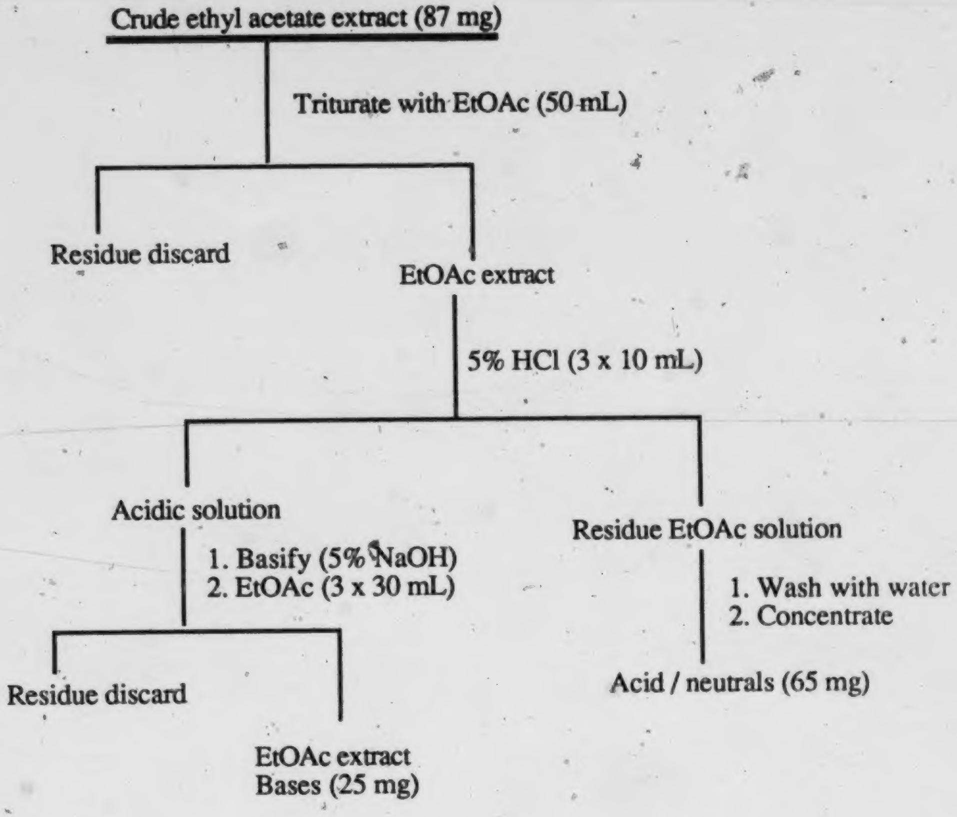
* All acetylations of the flavonoids were performed with excess Ac_2O and pyridine (1 mL). The solvent was concentrated in *vacuo* and the residue was purified by flash column chromatography.


Crude ethyl acetate extract (87 mg)
Triturate with EtOAc (50 mL)
Residue discard
EtOAc extract
5% HCl (3 x 10 mL)
Acidic solution
1. Basify (5% NaOH)
2. EtOAc (3 x 30 mL)
Residue EtOAc solution
1. Wash with water
2. Concentrate
Residue discard
EtOAc extract
Bases (25 mg)
Acid / neutrals (65 mg)

Scheme 28. Re-extraction of the crude bases of *E. umbellatum*.

Quercetin (77).

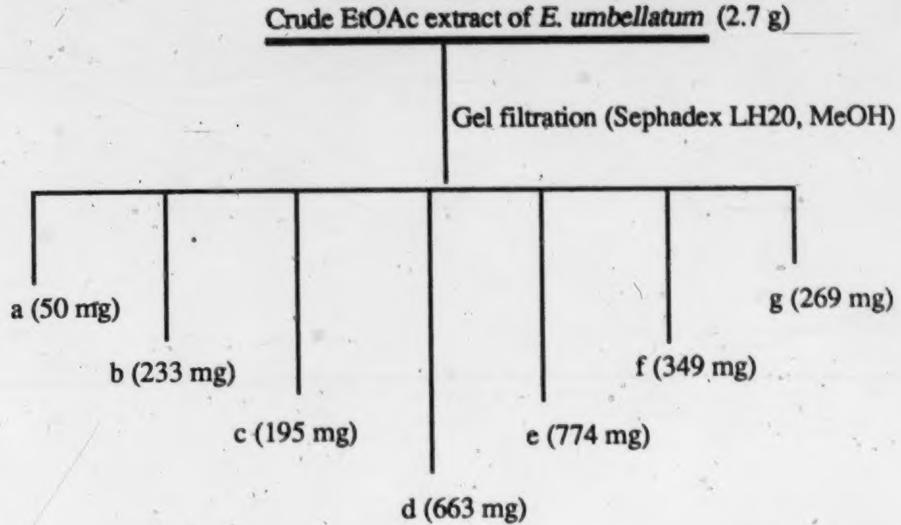
^{1}H nmr (200 MHz): 12.13 (1H, brs, OH-5), 8.0 (br, OH), 7.84 (1H, d, J = 2.2 Hz, H-2), 7.70 (1H, dd, J = 8.5,2.2 Hz, H-6'), 7.00 (1H, d, J = 8.5 Hz, H-5'), 6.52 (1H, d, J = 2 Hz, H-8), 6.27 (1H, d, J = 2 Hz, H-6); cims: m/z 303 (70, $M^{+}+1$); hreims: m/z calcd. for $C_{15}H_{10}O_7$: 302.0426, found, 302.0432 (36, M^{+}); pentaacetate (78), ftir υ_{max} cm^{-1}: 1771, 1650, 1621, 1191, 1174, 1145, 1124; ^{1}H nmr: see table 27.

Methylmyricetin (79).

^{1}H nmr (200 MHz): 12.18 (1H, brs, OH-5), 8.0 (br, OH), 7.45 (2H, s, H-2', H-6'), 6.68 (1H, d, J = 2 Hz, H-8), 6.32 (1H, d, J = 2 Hz, H-6); cims: m/z 333 (100, $M^{+}+1$); hreims: m/z calcd. for $C_{16}H_{12}O_8$: 332.0516, found: 332.0538 (33, M^{+}), pentaacetate (80), ftir υ_{max} cm^{-1}: 1776, 1640, 1620, 1120, 1186, 1150; ^{1}H nmr (200 MHz): 7.58 (2H, s, H-2', H-6'), 6.84 (1H, d, J = 2.5 Hz, H-8), 6.65 (1H, d, J = 2.5 Hz, H-6), 3.91 (3H, s, CH_3O-), 2.43 (3H, s, CH_3CO_2-), 2.36 (3H, s, CH_3CO_2-), 2.34 (3H, s, CH_3CO_2-), 2.33 (6H, s, 2 x CH_3CO_2-).

D3.4. Isolation of compounds 74, 81, 83, 85 and 87.

A portion of the ethyl acetate extract of *E. umbellatum* (2.7 g) was separated by gel filtration chromatography over Sephadex LH20 (70 cm x 20 mm column, methanol) into 7 fractions **a** to **g** (scheme 29). The fractions **c**, **e**, and **f** were further purified.


Crude EtOAc extract of *E. umbellatum* (2.7 g)
Gel filtration (Sephadex LH20, MeOH)
a (50 mg)
b (233 mg)
c (195 mg)
d (663 mg)
e (774 mg)
f (349 mg)
g (269 mg)

Scheme 29. Isolation of compounds **74**, **81**, **83**, **85**, and **87**.

Theogallin (3-O-galloylquinic acid) (74).

Theogallin (**74**) (76 mg) was obtained as a white solid from fraction c (184 mg) by flash column chromatography (chloroform:methanol:ethanol:water; 30:10:16:6), mp: 235°C (dec); tlc: Rf 0.16 (chloroform:methanol:ethanol:water; 30:16:16:6); ftir:υ_{max} cm^{-1}: 3600-2400 (br, OH), 1695, 1595, 1451, 1228, 1035; $[\alpha]_D = -37^o$ ($c = 0.19$, water); uv ($\underline{c} = 0.0048$, water) λ_{max} nm (logε): 278 (3.55); hreims: m/z calcd. for $C_{14}H_{16}O_{10}$: 344.0742; ^{1}H nmr (D_2O, 200 MHz): 6.90 (2H, s, galloyl H), 5.18 (1H, brddd, J = 10,10,4.5 Hz, H-3), 4.06 (1H, brd, J = 3 Hz, H-5eq), 3.74 (1H, brdd, J = 10, 3 Hz, H-4ax), 1.95 (4H, m, 2H-2,2H-6); ^{13}C nmr (D_2O-acetone-d_6, 75.5 MHz): 180.1 (s, $-\underline{C}O_2H$), 167.7 (s, $-\underline{C}O_2-$), 145.2 (s, 2 x C), 138.7 (s), 121.0 (s), 109 (d, 2 x

C), 76.9 (s, C-1), 73.2 (d, C-3), 72.2 (d, C-4)*, 71.3 (d, C-5)*, 39.0 (t, C-2)**, 37.7 (t, C-6)**.

Methylation of theogallin (74) with acidic methanol.

Hydrochloric acid (5%) was added dropwise to a solution of **74** (16 mg) in minimum water until pH 1-2. The solution was concentrated in *vacuo*, the residue redissolved in minimum methanol, and the methanol was concentrated in *vacuo*. The residue was purified by flash column chromatography (chloroform : methanol : ethanol : water; 30:16:6:6) to afford **75** (7 mg), tlc: Rf 0.57 (ethyl acetate:methanol:water; 12:3:1); ftir υ_{max} cm^{-1}: 3600-3100 (br, OH), 1727, 1705, 1608, 1230, 1031; ^{1}H nmr (MeOH-d_4, 400 MHz): 6.99 (2H, s, galloyl H), 5.28 (1H, ddd, J = 7,7,4 Hz, H-3ax), 4.16 (1H, ddd, J = 8,3,3 Hz, H-5eq), 3.78 (1H, dd, J = 7,3 Hz, H-4ax), 3.53 (3H, s, $C\underline{H}_3O_2C$-), 2.28 (1H, dd, J = 14,7 Hz, H-2ax), 2.17 (1H, dd, J = 13,3 Hz, H-6a), 2.15 (1H, dd, J = 14,4 Hz, H-2eq), 1.97 (1H, dd, J = 13,8 Hz, H-6b); ^{13}C nmr (MeOH-d_4, 75.5 MHz): 175.3 (s), 167.4 (s), 146.5 (s, 2 x C), 140.48 (s), 121.4 (s), 110.2 (d, 2 x C), 75.4 (s), 72.5 (d), 72.O (d), 69.8 (d), 52.9 (q), 37.9 (t), 37.O (t); cims: m/z 344 (8, (M^+-CH_3O) +1), hreims: m/z calcd. for $C_{15}H_{18}O_{10}$: 358.0899, found: 358.0870 (2, M^+), 340 ($C_{15}H_{16}O_9$, 1), 326 ($C_{14}H_{14}O_9$, 6), 170 ($C_7H_6O_5$, 21), 153 ($C_7H_5O_4$, 100), 125 ($C_6H_5O_3$, 10).

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* Signals may be reversed.
** " " " "

Methylation of theogallin (74) with diazomethane.

A solution of theogallin (**74**) (9.5 mg) in 98% ethanol was made acidic by dropwise addition of 5% HCl. Excess ethereal diazomethane was added and the mixture was stirred at room temperature for 15 minutes. The ether layer was separated and concentrated in *vacuo*. The residue was purified by flash column chromatography (5% methanol in chloroform) then pipette column chromatography (silica gel, 15% acetone in chloroform), to afford the tetramethoxyl derivative **76** (3.9 mg), tlc: Rf 0.28 (6% methanol in chloroform); ftir υ_{max} cm^{-1}: 3450, 1730, 1707, 1580, 1225, 1125; ^{1}H nmr (400 MHz): 7.30 (2H, s, galloyl H), 5.50 (1H, ddd, J = 12,10,5 Hz, H-3ax), 4.25 (1H, dq, J = 7,3 Hz, H-5eq), 4.05 (1H, brs, $W_{1/2}$ = 5.5 Hz, -O$\underline{H}$-1), 3.88 (9H, s, 3 x C$\underline{H}_3$O-), 3.80 (3H, s, C$\underline{H}_3$O-), 3.78 (1H, brddd, J = 10,10,3 Hz, H-4ax), 3.69 (1H, brd, J = 7 Hz, -O$\underline{H}$-5), 3.01 (1H, brd, J = 10 Hz, -O$\underline{H}$-4), 2.36 (1H, brddd, J = 12,5,3 Hz, H-2eq), 2.26 (1H, dt, J = 15,3 Hz, H-6eq), 2.13 (1H, dd, J = 15,3 Hz, H-6ax), 2.08 (1H, dd, J = 12,12 Hz, H-2ax); ^{13}C nmr (75.5 MHz): 174.5, 166.5, 153.0, 142.6, 124.8 (2 x C), 107.1 (2 x C), 75.7, 74.0, 71.6, 70.7, 61.00, 56.3(2 x C), 53.4, 38.8, 37.0; cims: m/z 418 (17, M$^+$+18); 401 (45, M$^+$+1), 386 (100, (M$^+$-CH$_3$O)+18); hreims: m/z calcd. for $C_{18}H_{24}O_{10}$: 400.1369, found: 400.1368 (30, M$^+$), 369 ($C_{17}H_{21}O_9$, 5), 368 ($C_{17}H_{20}O_9$, 28), 212 ($C_{10}H_{12}O_5$, 59), 195 ($C_{10}H_{11}O_4$, 100).

Methylmyricetin 3-O-rhamnoside (81).

Fraction **e** (730 mg) (scheme 29) was separated into 9 fractions **e-1** to **e-9** by flash column chromatography (ethyl acetate:butanone:water:formic:acid; 35:7:1:1). Relatively pure methylmyricetin 3-O-rhamnoside (48 mg) was obtained from fraction **e-5** (214.5 mg) by crystallization from methanol (a minor impurity was visible in the ^{1}H and ^{13}C nmr), ftir υ_{max} cm^{-1}: 3431-3217 (br, OH), 1661, 1598; $[\alpha]_D$ = -154° ($\underline{c}$ = 0.11, methanol); uv (c = 0.016, methanol) λ_{max} nm (log ε): 255 (3.66), *ca* 300 (shoulder), 353 (3.59); ^{1}H nmr (DMSO-d_6, 200 MHz): 12.8 (1H, s), 6.87 (2H, s, H-2',H-6'), 6.65 (1H, d, J = 2 Hz, H-8), 6.40 (1H, d, J = 2 Hz, H-6), 5.21 (1H, brs, $W_{1/2}$ = 3 Hz, H-1"), 4.12 (1H, brs, $W_{1/2}$ = 6.5 Hz, H-2"), 3.94 (3H, s, C̲H̲$_3$O-), 3.65 (1H, brdd, J = 9,3 Hz, H-3"), 3.47 (1H, m, H-5"), 3.25 (1H, dd, J = 9,9 Hz, H-4"), 0.99 (3H, d, J = 6 Hz); ^{13}C nmr: see table 26. Acetylation of **81** (5 mg) and purification* afforded the hexaacetate **82** (3.5 mg), ^{1}H nmr (400 MHz): see table 26; cims: m/z 748 (54, M$^+$+18), 476 (82, M$^+$ aglycone +18)), 290 (100, M$^+$ sugar + 18); hreims: m/z calcd. for $C_{34}H_{34}O_{18}$: 730.1744 (M$^+$), 458 ($C_{22}H_{18}O_{11}$, 10), 416 ($C_{20}H_{16}O_{10}$, 24), 374 ($C_{18}H_{14}O_9$, 19), 332 ($C_{16}H_{12}O_8$, 45), 273 ($C_{12}H_{17}O_7$, 29).

Myricetin 3-O-rhamnoside (83), myricetin 3-O-glycoside (85) and quercetin 3-O-glycoside (87).

Fraction f (323 mg) was separated into 7 fractions **f-1** to **f-7** by slow column chromatography over cellulose previously equilibrated with ethyl acetate and using benzene:ethyl formate:formic acid:water; 9:21:6:5 as eluant. Myricetin 3-O-rhamnoside (**83**) (37 mg) and quercetin 3-O-glycoside (**85**) (32 mg) was obtained from fraction **f-4** (127 mg) by flash column chromatography (ethyl acetate:butanone:formic acid:water; 20:4:1:1). Myricetin 3-O-glycoside (**87**) (38 mg) were obtained from fraction **f-7** (97 mg) by flash column chromatography (ethyl acetate:butanone:formic acid:water; 12:3:1:1). Compounds **83**, **85**, and **87** were acetylated and characterized as the acetates **84**, **86**, and **88**, respectively.

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* All purifications of the acetylated mixtures of flavonol glycosides were performed by flash column chromatography using 30% ethyl acetate in chloroform as eluant.

2",3',3",4',4",5,5',7-Octaacetylmyricetin 3-O-rhamnoside (84).

tlc: Rf 0.25 (ethyl acetate:chloroform; 3:7); ftir υ_{max} cm^{-1}: 1780, 1750, 1647, 1628, 1434, 1371, 1216, 1185; $[\alpha]_D$= -167° (c = 0.15, methanol); ^{1}H nmr: see table 26; ^{13}Cnmr (100.6 MHz): 172.2, 170.1, 169.6, 169.4, 169.0, 167.7, 167.1(2 x C), 166.0, 156.7, 154.2, 153.3, 150.6, 144.1 (2 x C), 137.3, 137.2, 128.0, 121.2 (2 x C), 115.2, 113.7, 108.7, 98.2, 77.2, 70.5, 69.2, 68.8, 21.0, 20.9, 20.7, 20.5, 20.4 (3 x C), 20.0, 17.0.

2",3',3",4',4",5,5",7-Octaacetylquercetin 3-O-glycoside (86).

tlc: Rf 0.25 (ethyl acetate:chloroform; 3:7); ftir υ_{max} cm^{-1}: 1777, 1664, 1628, 1435, 1371, 1264, 1215; $[\alpha]_D$= -88° ($\underline{c}$ = 0.22, methanol); ^{1}H nmr (200 MHz): 7.98 (1H, dd, J = 9,2 Hz, H-6'), 7.92 (1H, d, J = 2 Hz, H-2'), 7.33 (1H, d, J = 9Hz, H-5'), 7.32 (1H, d, J = 2 Hz, H-8), 6.84 (1H, d, J = 2 Hz, H-6), 5.48 (1H, d, J = 8 Hz, H-1"), 5.39-5.30 (2H, m), 5.07(1H, dd, J = 10,3 Hz), 3.76-3.93 (3H, m), 2.45 (3H, s, $C\underline{H}_3CO_2$-), 2.35 (6H, s, 2 x $C\underline{H}_3CO_2$-), 2.32 (3H, s, $C\underline{H}_3CO_2$-), 2.13 (3H, s, $C\underline{H}_3CO_2$-), 2.00 (3H, s, $C\underline{H}_3CO_2$-); 1.92 (3H, s, $C\underline{H}_3CO_2$-); ^{13}C nmr (100.6 MHz): 172.0, 170.2, 169.9 (2 x C), 169.0, 167.8(2 x C), 156.7, 154.5, 154.1, 150.4, 142.1, 136.9, 128.8, 127.4, 124.5,123.2, 113.5, 108.8, 99.6, 71.2, 70.9, 69.3, 67.0, 60.9, 21.0, 21.0, 20.8, 20.6, 20.5, 20.4 (2 x C).

2",3',3",4',4",5,5',5",7-nonaacetylmyricetin 3-O-glycoside (88).

tlc: Rf 0.2 (ethyl acetate:chloroform; 3:7); ftir υ_{max} cm^{-1}: 1779, 1752, 1645, 1628, 1370, 1218, 1135; $[\alpha]_D$= -72° ($\underline{c}$ = 0.12, methanol); ^{1}H nmr (200 MHz): 7.87 (2H, s, H-2',H-6'), 7.33 (1H, d, J = 2 Hz, H-8), 6.85 (1H, d, J = 2 Hz, H-6), 5.56

(1H, d, J = 8 Hz, H-1"), 5.41-5.32 (1H, m), 5.09 (1H, dd, J =10,3 Hz), 4.01-3.77 (3H, m), 2.44 (3H, s, $C\underline{H}_3CO_2$-), 2.34 (9H, s, 3 x $C\underline{H}_3CO_2$-), 2.31 (3H, s, $C\underline{H}_3CO_2$-), 2.16 (3H, s, $C\underline{H}_3CO_2$-), 2.14 (3H, s, $C\underline{H}_3CO_2$-), 2.00 (3H, s, $C\underline{H}_3CO_2$-), 1.91 (3H, s, $C\underline{H}_3CO_2$-); ^{13}C nmr (100.6 MHz): 170.3 (s), 170.0 (s), 169.8 (s), 169.8 (s, 2 x C), 16.8.9 (s), 167.7 (s), 167.3(s, 2 x C, 166.2 (s), 156.6 (s), 154.2 (s), 153.6 (s), 150.4 (s), 143.5 (s, 2 x C), 128.4 (s), 121.6 (d, 2 x C), 113.5 (d), 108.8 (d), 99.5 (d), 71.4 (d), 70.9 (d), 69.3 (d), 67.0 (d), 61.0 (t), 21.0 (q, 2 x C), 20.9 (q), 20.7 (q), 20.5 (q, 3 x C), 20.4 (q), 20.3 (q); cims: m/z 546 (100, M$^+$ aglycone +18), 331 (93, M$^+$ sugar); lreims: m/z 470 (9), 428 (5), 386 (14), 368 (7), 344 (12), 302 (10).

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